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Oral Presentations



Acinetobacter 2019



Opening Lecture:

***Acinetobacter baumannii*: from the clinical management to the non-antimicrobial approaches**

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Acinetobacter baumannii emerged as a widespread hospital-acquired pathogen in the nineties of the XX Century, producing endemic and/or outbreaks infections, mostly in the intensive care units, as ventilator-associated pneumonia (VAP) or bacteraemia. Moreover, in the high colonization prevalence setting, infections also occurred in surgical wards, producing meningitis, skin and soft-tissue infections, surgical-site infections, and bacteraemia from different sources, including intravenous catheters. Although infections in the community are scarce, some virulent strains may produce community-acquired pneumonia or specific situations as wound wars have been reported. Many efforts were made to better understand the clinical and molecular epidemiology and the risk factors associated to hospital infections, to implement effective preventive measures, which have been only successful when a bundle of measures and a very well coordinated prevention control team have worked in a long and hard way. Multidrug-resistant (MDR) and extensively-drug resistant (XDR) strains appeared shortly after the endemic of *A. baumannii* infections. Thus, resistance to carbapenems, which were the gold-standard therapy, besides resistance to most of the antimicrobial families, including beta-lactams, quinolones, aminoglycosides, and tetracyclines are very common in many countries. Polymyxins were the last drug to treat many patients, but without optimal cure rates, and with dosage depending on the MIC of the strain producing the infection. Some works were carried out to improve the pharmacodynamics of colistin using load doses or using aerosolized or intraventricular colistin in VAP and neurosurgical meningitis, respectively, with the aim to improve the clinical outcome. Antimicrobial combinations have been also tested in experimental infections models, especially with rifampicin. However, a quick appearance of rifampicin-resistance followed its introduction in the clinical practice. It must be underline that *A. baumannii* severe infections have a clinically important morbidity and mortality rates, including when the antimicrobials are active against the strains producing the infections. This fact, together with the frequent MDR/XDR infections, prompted the research on the therapeutic efficacy of non-antimicrobial approaches, such as immunomodulatory molecules, peptides targeting virulence factors, immunotherapy, and the repurposing of molecules as tamoxifen or drugs for helminthiasis.

Keynote Lecture 1:

Tracking Lineages of Extensively Antibiotic Resistant GC1 and GC2 *A. baumannii*

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In most countries, *Acinetobacter baumannii* strains resistant to many different antibiotics are associated with a few globally disseminated clones, and the GC1 (CC1) and GC2 (CC2) clones have been dominant since the late 1970s. However, many different GC1 and GC2 lineages have evolved over the decades, each derived from a single cell that acquired a novel feature and some have subsequently circulated globally. To understand and track the spread of these important clones, an understanding of the evolution of these lineages and the relationships between them is needed. We are exploring the evolution of the GC1 and GC2 clonal complexes using de-novo assemblies of genomes sequenced using short read data coupled with completed reference genomes. Resistance gene content and resistance mutations are assessed and chromosomal resistance islands are assembled using PCR. Plasmid sequences are retrieved via matches with known plasmids and complete plasmid sequences assembled and tested for mobility. Some lineages can be defined by variations in the identity and gene content of the chromosomal resistance islands, and carbapenem or aminoglycoside resistance genes have been acquired repeatedly generating a multitude of further sub-lineages. Extracellular polysaccharides, which are critical virulence factors, also vary due to extensive within clone variation in the gene content at the K (capsule biosynthesis) and OC (outer core of lipooligosaccharide) loci providing further lineage markers. Recently, locations of ISAbal insertion sequences have been identified using ISMapper and ISSeeker and verified in a reference by PCR or long read sequencing. The locations of shared ISAbal correlate well with standard phylogenies, and using ISAbal locations we have deduced evolutionary pathways in each clone as a whole and within specific lineages. However, all of these variations provide highly discriminatory markers for outbreak detection and can help to track local and international spread.

O1-1: Allelic variation of *pmrCAB* operon is associated with *Acinetobacter baumannii* international clonal lineages and represents a genomic recombination site

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One of the reasons for the success of *Acinetobacter baumannii* (*Ab*) as an opportunistic human pathogen is its high genomic plasticity and its ability to acquire resistance determinants [1,2]. Mutations in *pmrCAB* are the main mechanism causing reduced susceptibility to colistin in *Ab* [3]. However, most studies rely on comparison of gene sequences against reference strains to determine the presence of non-synonymous mutations in colistin-resistant *Ab* (ColR-*Ab*) isolates. Moreover, such a mechanism has not been considered horizontally transferable. In this study, we evaluated the allelic variation of *pmrCAB* genes in different *Ab* lineages.

114 *Ab* isolates belonging to international clones (IC) 2 (n=65), 4 (n=24), 5 (n=18), and 7 (n=7) had their genomes sequenced using MiSeq. Genomes were assembled using Velvet and *pmrCAB* sequences were aligned to identify allelic variations. Sequences belonging to *Ab* reference strains ATCC 19606, ATCC 17978, AYE, and ACICU were used for comparison.

The *pmrCAB* alleles were conserved within each *Ab* clonal lineage. The *pmrC* gene exhibited the highest variability between ICs, showing up to 20 non-synonymous mutations between each allele. On the other hand, *pmrA* was the most conserved allele, as only isolates belonging to IC4 presented substitutions compared to reference strains. Lastly, *pmrB* seems to be a hotspot for substitutions associated with reduced susceptibility to colistin, as most mutations in this gene were observed among ColR-*Ab* isolates, regardless to which clonal lineage they belonged. Interestingly, some isolates belonging to IC2 and IC7 presented *pmrCAB* alleles associated with IC4, suggesting that this operon is within a recombination site. In addition, this phenomenon was observed in both colistin-susceptible and -resistant isolates, suggesting that colistin pressure does not drive recombination. Finally, such genetic events could indicate that colistin resistance in *Ab* might be acquired through recombination.

In conclusion, the *pmrCAB* operon is lineage-specific and such genetic variation should be considered when investigating colistin resistance in *Ab*. Moreover, this operon might be transferred from ColR-*Ab* isolates to other clonal lineages, jeopardizing the use of this antimicrobial agent as a last resort for treatment of serious infections caused by this pathogen.

[1] Peleg *et al.*, *CMR*, **21**:538-82, 2008

[2] Antunes *et al.*, *Pathog Dis*, **71**:292-301, 2014

[3] Arroyo *et al.*, *AAC*, **55**:3743-51, 2011

O1-2: The evolutionary trajectory of *Acinetobacter* pathobiology

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Acinetobacter baumannii is responsible for an increasing number of both hospital- and community-acquired infections worldwide [1,2]. The clinical success of this bacterium can be attributed to its remarkably high genomic plasticity in combination with the acquisition of multi- and even pandrug resistance [3]. With novel antibiotics becoming steadily harder to obtain, a comprehensive understanding of how the pathogen adapts to, and interacts with, the human host is key to identifying new druggable targets.

Here, we present a comprehensive survey of genomic innovations that underlie *Acinetobacter* pathobiology. We integrate comparative phylogenomics considering the full currently known diversity of *Acinetobacter spp.* with extensive phylogenetic profiling to reconstruct the phylostratigraphy of the *Acinetobacter* pan-genome at an unprecedented resolution. On this basis, we investigate gain and loss of genes and of their corresponding functions along the *Acinetobacter* phylogeny. Moreover, we trace – for the first time – the lineage-specific functional modification of proteins encoded by evolutionary old genes as indicated by a change of the domain architecture. Our results reveal that, next to gene clusters involved in various metabolic pathways, particularly such genes are characteristic for the ACB clade that are involved into signaling and stress tolerance. As one example, AbaR and AbaI, two quorum sensing genes important for biofilm development, were gained by the last common ancestor of the ACB clade and are nowadays almost ubiquitously present in this clade. Rather unexpectedly, we found that the repertoire of *Acinetobacter* genes that mediate the interaction with the bacterial environment is evolutionarily old and is showing little evidence for lineage-specific changes in the pathogenic members of the ACB clade. This picture changes, however, substantially when increasing the focus to the comparative analysis of protein domain architectures. We provide evidence that pathogenic *Acinetobacter* adapt the interaction with their environment and with their host preferably by changing the function of existing genes rather than by modifying the gene repertoire itself. We provide a first level experimental curation of our novel virulence factor candidates by assessing their expression during host infection.

[1] Dijkshoorn, L., Nemec, A., & Seifert, H. (2007). An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*. *Nature Reviews. Microbiology*, 5(12), 939–51. <http://doi.org/10.1038/nrmicro1789>

[2] Peleg, A. Y., Seifert, H., & Paterson, D. L. (2008). *Acinetobacter baumannii*: emergence of a successful pathogen. *Clinical Microbiology Reviews*, 21(3), 538–82. <http://doi.org/10.1128/CMR.00058-07>

[3] Imperi, F., Antunes, L. C. S., Blom, J., Villa, L., Iacono, M., Visca, P., & Carattoli, A. (2011). The genomics of *Acinetobacter baumannii*: insights into genome plasticity, antimicrobial resistance and pathogenicity. *IUBMB Life*, 63(12), 1068–74. <http://doi.org/10.1002/iub.531>

O1-3: Structure and evolution of *Acinetobacter baumannii* plasmids

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Acinetobacter baumannii is an emergent bacterial pathogen that provokes many types of infections in hospitals around the world [1]. The genome of this organism consists of a chromosome and plasmids. These plasmids vary over a wide size range and many of them have been linked to the acquisition of antibiotic-resistance genes. To analyse the structure of the *A. baumannii* plasmids we used the 158 complete plasmid sequences deposited in NCBI as of August 14, 2017. However, considering that most of these plasmid sequences were obtained from isolates of international clones and/or from a restricted set of countries, we incorporated the sequences of 18 plasmids obtained from the genome sequences of 10 strains that represent some of the most prevalent STs circulating in Mexico to increase the plasmid diversity included in our investigation. We compared all plasmids of our collection using MEGABLAST. To analyze these results results, we constructed networks with the following rule: two plasmids are linked if at least 85% of the regions of the largest plasmid (for each comparison) are covered by the smaller plasmid, and those regions exhibit at least 90% identical coverage. We found that the 128 *A. baumannii* plasmids of our collection were organized into 23 groups, and 47 plasmids remained as orphans. Interestingly, 80 plasmids (45.7%) were clustered in only four groups. The other 19 groups are very small, as most of them contain only two members. Our results indicate that *A. baumannii* plasmids belong to a small number of plasmid lineages. The general structure of these lineages seems to be very stable and consists not only of genes involved in plasmid maintenance functions but of gene sets encoding poorly characterized proteins, not obviously linked to survival in the hospital setting, and opening the possibility that they improve the parasitic properties of plasmids. An analysis of genes involved in replication, strongly suggests that each plasmid lineage represent a plasmid incompatibility group. The same analysis showed the necessity of classifying the Rep proteins in ten new groups, under the scheme proposed by Bertini and co-workers (2010)[2]. Also, we show that some plasmid lineages have the potential capacity to replicate in many bacterial genera including those embracing human pathogen species, while others seem to replicate only within the limits of the *Acinetobacter* genus. Moreover, some plasmid lineages are widely distributed along the *A. baumannii* phylogenetic tree. Despite this, a number of them lack genes involved in conjugation or mobilization functions. Interestingly, only 34.6% of the plasmids analysed here possess antibiotic resistance genes and most of them belong to thirteen plasmid lineages of the twenty-three described here. Gene flux between plasmid lineages seems to be limited essentially to transposable elements sometimes linked to antibiotic resistance genes.

[1] L. Dijkshoorn, A. Nemeč and H. Seifert. An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*. *Nat. Rev. Microbiol.* **5** (2007), 939-951.

[2] A. Bertini, L. Poirel, P.D. Mugnier, L. Villa, P. Nordmann, A. Carattoli. Characterization and PCR-based replicon typing of resistance plasmids in *Acinetobacter baumannii*. *Antimicrob Agents Chemother.* (2010) 54(10):4168-4177.

O1-4: DNA single-strands cause microindel mutations

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Intracellular DNA single-strands are a genomic damage signal and their occurrence is tightly controlled in cells under benign environmental conditions. In rare cases, single-stranded DNA molecules can act as mutagens and cause SNP clusters or microindels of highly variable sequences and sizes (three to >90 bp). These mutations are rare ($<10^{-12}$ per cell and locus) and occur by annealing of the single-strands at replication forks or gaps with genomic DNA, acting as primers for Okazaki fragments or for fill-in synthesis. The annealing occurs at one or more microhomologies (short stretches of complementary nucleotides in heterologous DNA), initiating illegitimate recombinant joints at the annealed loci. The mechanism has been termed short-patch double illegitimate recombination (SPDIR).

SPDIR mutations are elevated under genotoxic stress and in genome maintenance mutants (lacking single-strand-specific nucleases or single-stranded DNA binding proteins such as DprA and RecA). In a *recA recJ exoX* triple mutant, SPDIR mutations are increased >7,000-fold. The resulting microindel mutations are caused by DNA from random loci in the bacterial genome.

Moreover, *A. baylyi* can actively take up any DNA in the course of natural transformation. The DNA is transported into the cytoplasm as single-strands, and these molecules can also rarely cause microindel mutations. Foreign DNA can increase the variability of SPDIR mutations considerably. However, DNA sequence analyses show that even when donor DNA is present in excess, most SPDIR mutations are formed with cognate DNA, confirming that SPDIR is mainly an intragenomic process.

SPDIR mutations typically cause complex changes in a single generation that are not achievable by point mutations or genomic rearrangements, allowing cells to deeper explore the fitness landscape. Bioinformatic studies reveal that SPDIR mutations occur in prokaryotes and eukaryotes, including humans, and may be involved in cancer progression.

Keynote Lecture 2:

Iron and *Acinetobacter baumannii* pathogenicity

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Acinetobacter baumannii is a multidrug-resistant nosocomial pathogen. During infection, *A. baumannii* responds to low iron availability imposed by the host through the exploitation of multiple iron-acquisition strategies. To date, six different gene clusters for active iron uptake have been described in *A. baumannii* strains, some of which are variably present, and encode protein systems involved in ferrisiderophore, heme and ferrous iron transport. We demonstrated that iron is essential for *A. baumannii* pathogenicity, since a knock-out in the TonB3 energy-transducing system, which impairs both ferrisiderophore and heme uptake, completely abrogates *A. baumannii* lethality in different animal models of infection. Therefore, TonB3 can be regarded as a suitable candidate target for the development of new antimicrobial compounds. Iron metabolism was also exploited as a druggable target. We and others showed that the iron mimetic metal gallium (Ga) disrupts ferric iron-dependent metabolic pathways, thereby inhibiting microbial growth. Among gallium compounds tested, Ga-protoporphirin IX showed extremely high activity against some *A. baumannii* strains (MIC 0.25-1.0 μM), dependent on the presence of a particular heme-uptake systems. Moreover, Ga^{3+} inhibited both planktonic and biofilm *A. baumannii* growth. These findings raise hope for the future development of new antimicrobial compounds capable of interfering with *A. baumannii* iron transport and metabolism.

O2-1: Impact of the *Acinetobacter* Trimeric Autotransporter Adhesin Ata on Pathogenicity of *Acinetobacter baumannii* and the Host Cell Response

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The outer membrane protein Ata of *Acinetobacter baumannii* belongs to the superfamily of trimeric autotransporter adhesins which are important virulence factors in many Gram-negative bacteria. To investigate the impact of Ata on the host cell response upon infection, human umbilical cord vein endothelial cells (HUVECs) were infected with *A. baumannii* ATCC 19606 (WT) or an isogenic *ata* knock-out strain (Δ *ata*).

Quantitative proteomics employing hybrid liquid chromatography/mass spectrometry (LC/MS) was carried out to analyze protein levels of HUVECs 14 h post infection. A total of 4,969 human proteins were quantified, from which 106 (WT) and 51 (Δ *ata*) proteins were differentially produced (fold change \geq 2.0; false discovery rate $<$ 0.05). Two signaling pathways involved in inflammation/immune response and initiation of apoptosis were identified and the involved proteins were further verified in infection experiments.

Quantification of pro-inflammatory cytokines of infected HUVECs using an ELISA array revealed that interleukin-8 and interleukin-6 were secreted in a time- and Ata-dependent manner. Likewise, chemotaxis of human THP-1 monocytes of infected HUVECs in a transmigration assay was significantly increased upon infection with the WT compared to Δ *ata*. After injection of *Galleria mellonella* larvae, *ata* deletion mutants were attenuated in their ability to recruit immune cells *in vivo*.

To determine the role of Ata in apoptosis, propidium iodide/annexin V staining with subsequent FACS analysis and caspase activation assays were employed. Infection of HUVECs with *A. baumannii* WT was associated with higher apoptosis rates via cleavage of caspase-3 and caspase-7, but not necrosis, in comparison to Δ *ata*. Furthermore, the production of clusterin, a negative regulator of apoptosis, was downregulated 3.7-fold upon infection with the WT, indicating an enhanced apoptosis rate, while production of clusterin was not significantly influenced when using the Δ *ata* strain.

In summary, Ata caused a pro-inflammatory host cell response *in vitro* and *in vivo* and induced apoptosis by activation of caspase-3/-7. These results strongly suggest that Ata is an important multifunctional virulence factor of *A. baumannii* involved in interaction with the host cell.

O2-2: The *Acinetobacter baumannii* outer membrane protein CarO exhibits virulence factor roles in a murine infection model

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Infections due to multi-drug resistant *Acinetobacter baumannii* are associated with high morbidity and mortality among critically-ill and immunocompromised patients. Novel approaches to prevent and treat these infections are needed, and inhibitors blocking virulence factors are promising alternatives to antimicrobials for the treatment of pan-drug resistant strains [1, 2]. Outer membrane (OM) proteins functioning at the interface with the environment constitute prime candidates for the design of inhibitors aimed to disturb recognition of target cells by the pathogen [1, 2]. Here, we evaluated the potential roles of CarO, an OM protein found only among *Moraxellaceae* family members [3], in *A. baumannii* virulence. Wild-type *A. baumannii* strain ATCC 17978 (WT), its isogenic *carO* deletion mutant (*DcarO*), and the *DcarO* strain complemented with plasmid pWH1266-*carO* expressing CarO were used. Using an experimental murine model of peritoneal sepsis and C57BL/6 female mice, the minimum lethal dose for the WT strain and the *DcarO* mutant were 3.20 log₁₀ CFU/mL and 4.32 log₁₀ CFU/mL, respectively. Using an inoculum of 3.2 log₁₀ CFU/mL, all mice infected with the WT strain died within 24 h, 67% of mice infected with the *DcarO* mutant within 48 h, and all mice infected with the complemented strain within 24-48 h. Mice infected with the *DcarO* mutant showed significantly less bacterial burden at 8 h and 24 h-post infection in spleen, lung, kidney, liver, peritoneal fluid, and blood when compared with animals infected with the WT strain or the complemented *DcarO* strain. The overall results indicate that the loss of CarO significantly reduces, but not abolishes, the pathogenicity of the ATCC17978 strain in a murine model, pointing to this OM protein as part of the *A. baumannii* virulence repertoire and potential target for drug design.

[1] R. López-Rojas, Y. Smani, and J. Pachón. Treating multidrug-resistant *Acinetobacter baumannii* infection by blocking its virulence factors. *Expert Rev Anti Infect Ther.* **11** (2013): 231-233.

[2] X. Vila-Farrés, R. Parra-Millán, V. Sánchez-Encinales, *et al.* Combating virulence of Gram-negative bacilli by OmpA inhibition. *Sci Rep.* (2017) **7**:14683.

[3] M.A. Mussi, A.S. Limansky, V. Relling, *et al.* Horizontal gene transfer and assortative recombination within the *Acinetobacter baumannii* clinical population provide genetic diversity at the single *carO* gene, encoding a major outer membrane protein channel. *J Bacteriol.* (2011) **193**:4736-4748.

O2-3: CpaA, a novel secreted protease of *Acinetobacter* spp., targets host glycoproteins

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A. baumannii has recently emerged as one of the most concerning Gram-negative pathogens to infiltrate the hospital setting. New strategies for treating and managing multidrug-resistant *A. baumannii* infections are urgently needed, but a detailed understanding of this organism's pathobiology is required. Type II secretion system (T2SS) is required for full virulence in *Acinetobacter* spp. Of particular interest is the metallo-endopeptidase CpaA. CpaA is the most abundantly secreted T2SS effector, and its expression and secretion is conserved among recent medically-relevant *Acinetobacter* species. Deletion of *cpaA* results in attenuation of *Acinetobacter* spp. virulence in several infection models and impaired growth in urine. CpaA is the first bona fide secreted virulence factor in *Acinetobacter*; thus, understanding the role of CpaA in pathogenesis may lead to novel treatments for *Acinetobacter* infections. CpaA secretion is dependent on CpaB, a highly specific membrane-bound chaperone. We employed a biochemical and structural approach to further characterize the CpaA-CpaB interaction. We obtained the X-ray crystal structure of the CpaA-CpaB complex. Our results show that CpaA and CpaB strongly interact. Structural analysis revealed that CpaA contains four oligosaccharide binding (OB)-fold domains, not predicted from the primary sequence, probably to target glycoproteins. We demonstrate that CpaA recognizes a variety of human glycoproteins, such as human Factor V and mucins.

O2-4: Potential mechanisms of mucin-enhanced *Acinetobacter baumannii* virulence in the mouse model of intraperitoneal infection

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Porcine mucin has been commonly used to enhance the infectivity of bacterial pathogens including *Acinetobacter baumannii* in animal models, but the mechanisms for enhancement *in vivo* by mucin remain relatively unknown. Using the mouse model of intraperitoneal (i.p.) mucin-enhanced *A. baumannii* infection, we characterized the kinetics of bacterial replication and dissemination and the host innate immune responses, as well as their potential contribution to the mucin-enhanced bacterial virulence. We found that mucin, either admixed with or separately injected with the challenge bacterial inoculum, was able to enhance the tissue and blood burdens of *A. baumannii* strains of different virulence. Intraperitoneal injection of *A. baumannii*/mucin or mucin alone induced a significant, but comparable reduction of peritoneal macrophages and lymphocytes, accompanied with a significant neutrophil recruitment and early interleukin (IL)-10 responses, suggesting that the resulted inflammatory cellular and cytokine responses were largely induced by the mucin. Depletion of peritoneal macrophages or neutralization of endogenous IL-10 activities showed no effect on the mucin-enhanced infectivity. However, pre-treatment of mucin with iron chelator DIBI, but not deferoxamine, partially abolished its virulence enhancement ability and substitution of mucin with iron significantly enhanced the bacterial burdens in peritoneal cavity and lung. Taken together, our results favour the hypothesis that iron is at least partially contributing to the mucin-enhanced infectivity of *A. baumannii* in this model, and emphasized the critical role of pathogens in determining the complexity of the mechanisms of mucin-enhanced bacterial virulence. Our findings also raise the possibility to use iron supplementation to develop a more defined and easier to standardize mouse model of i.p. *A. baumannii* infection.

Keynote Lecture 3:

Role of rapid diagnostics in prevention and control of multidrug-resistant *Acinetobacter*

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Multidrug resistance is commonly encountered in *Acinetobacter baumannii*. Treatment often relies on last-resort antibiotics including carbapenems and colistin. Rapid detection of resistance to those molecules therefore constitutes a challenge for clinical microbiologists in order to prevent deleterious individual and collective consequences as (i) delaying efficient antibiotic therapy that worsens the survival of the most severely-ill patients, or (ii) delaying isolation of carriers of multidrug-resistant bacteria and promoting outbreaks. From simple cheap biochemical test to whole-genome sequencing, clinical microbiologists must select the most adequate phenotypic and genotypic tools to promptly detect and antibiotic resistance from cultivated bacteria or from clinical specimens. Many different technical approaches can be used for these purposes, including molecular, biochemical and immunologic assays. The continuous implementation of newly-developed diagnostic techniques in clinical laboratories is crucial in order to detect, control, and therefore prevent dissemination of multidrug-resistant bacteria.

O3-1: Emergence of Colistin Resistance among Carbapenemase-Producing *Acinetobacter baumannii* Clinical Isolates from Egypt

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The emergence of colistin-resistant *A. baumannii* is a serious public health problem as it limits the treatment options against this opportunistic pathogen. The current study investigated the emergence of colistin resistance among carbapenem-resistant *A. baumannii* (CRAB) isolates in Egypt by several phenotypic and genetic methods. A total of 22 clinically recovered *A. baumannii* isolates from hospitalized patients were identified, and screening of their antimicrobial susceptibilities was performed using the VITEK-2 system. MICs for colistin susceptibility were determined using broth microdilution (BMD), agar dilution tests, VITEK-2 (GN222) and E-test. Characterization of carbapenem and colistin resistance determinants was performed using PCR and whole genome sequencing (Illumina MiSeq). Phenotypic testing showed that 9 of 22 isolates (40.9%) were colistin resistant by BMD and 7 of them were resistant by agar dilution, with an overall agreement between the 2 tests in 16/22 isolates (72.7%). Reliability of phenotypic tests in delineating colistin breakpoints was poor: E-test and VITEK-2 showed high very major error rates (40.9%), agar dilution (9%) compared to BMD with lack of categorical agreement between them. PCR results revealed that all isolates carried *bla*_{OXA-51-like} genes whereas *bla*_{OXA-23-like} was detected in 64% (14/22) and *bla*_{NDM-1} in 27% (6/22). Only one isolate harboured *bla*_{GES-like}. Whole genome sequence analyses performed on 7 strains suggests that colistin resistance could be attributed to multiple mutations in *pmrABC* genes. Furthermore, genome analysis revealed six isolates belonged to international clone 2 (IC) of *A. baumannii* while the remaining strain was sporadic as it did not cluster with any of the international clones. In conclusion the emergence of colistin resistance among CRAB clinical isolates in Egypt is alarming which further limits therapy options and requires prudent antimicrobial stewardship. The high incidence of the high-risk lineage IC2 harbouring *bla*_{OXA-23} as well as *bla*_{NDM} is also of concern. BMD is the only reliable phenotypic method for detection of colistin resistance and the resistance mechanisms need to be further elucidated.

O3-2: Characterization of induced antibiotic tolerance in *Acinetobacter baumannii*

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Acinetobacter baumannii is an opportunistic bacterial pathogen that has been designated as one of the six ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* species) pathogens of greatest healthcare concern. Although the organism is capable of causing a variety of infections, the most common manifestations are infections of the blood and lungs. Interestingly, otherwise clinically defined antibiotic susceptible *A. baumannii* bloodstream and lung infections frequently do not respond to therapy resulting in corresponding high rates of morbidity and mortality. To that end we have previously shown that *A. baumannii* upregulates numerous drug efflux pumps and demonstrates corresponding antibiotic tolerance when cultured in human serum, suggesting a mechanism for antibiotic failure in the clinic. We have also demonstrated induced antibiotic tolerance in lung surfactant, presumably via a similar mechanism. Although data suggests efflux as a possible explanation, the exact mechanisms of induced antibiotic tolerance are not well understood. To address this, we have employed two approaches: a genetic approach as well as a chemical genomics approach via use of small molecules that result in the loss of serum-induced tolerance. A transposon mutant library analyzed for serum grown cells that are no longer antibiotic tolerant has revealed potential genetic determinants of induced antibiotic tolerance, including a putative Resistance-Nodulation Division (RND) efflux pump as well as a transcription factor implemented in bacterial stress responses. In addition, we have identified a novel putative efflux pump inhibitor that reverses host-milieu associated antibiotic tolerance and potentiates antibiotics in both serum and lung surfactant. Furthermore, a series of structural analogs have been generated for structure-activity relationship studies and have been analyzed for efflux inhibition, membrane permeabilization and antibiotic potentiation in both serum and lung surfactant.

O3-3: Comparative characteristics of OXA-23/IC2, OXA-23/IC1, OXA-58/IC1 and OXA-58/IC2 carbapenem-resistant *Acinetobacter baumannii*

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Carbapenem-resistant *Acinetobacter baumannii* (CRAB) have become endemic in many European countries. Currently, CRAB isolates predominantly belong to International Clone (IC) 2 and produce OXA-23 carbapenemase. This predominance might be driven by selective advantages of the IC2 OXA-23 producers. Although OXA-23 exhibits stronger carbapenemase activity and OXA-23 producers have higher carbapenem MICs, relative to the OXA-58 producers, the reasons for the shift to the epidemic IC2/OXA-23-producing strains seem to be more complex. We investigated and compare by whole-genomic and phenotypic methods OXA-58-producing CRAB of IC1 and IC2 and OXA-23-producing CRAB of IC1 and IC2 from six European countries.

We studied fifty-two single-patient non-repetitive clinical CRAB, representative of the four groups. MICs to colistin, tigecycline, rifampicin, meropenem, and imipenem were determined by broth microdilution. Growth rates were compared between groups using Student's t-test. Other phenotypic characteristics assayed were biofilm formation, resistance to human serum, iron and heme uptake, inducible lytic phage and bacteriocin sensitivity. Whole genome sequencing (WGS) was performed in Illumina platform. Draft genomes were de novo assembled using SPAdes via INNUca and annotated with Prokka. ARG and virulence factors were searched with Abricate.

IC2/OXA-23 isolates were resistant (or non-susceptible) to more antibiotics than other groups. Also, IC2/OXA-23 isolates were more resistant to bacteriocins than other groups. No significant advantage was found for IC2/OXA-23 regarding human serum resistance, heme uptake, biofilm production, siderophores production and heme uptake. WGS-derived phylogenetic tree revealed two main genetic lineages, one comprising IC1 CRAB (carrying either OXA-23 or OXA-58) and a 2nd comprising IC2 isolates. In the latter group, a substructure was observed with IC2/OXA-58 and IC2/OXA-23 forming two sub-lineages.

Analysis of this representative collection of CRAB from Europe showed that the currently predominant IC2/OXA-23 CRAB form a distinct sub-lineage. The detected traits that might favor the selection of this sub-lineage are non-susceptibility to colistin, minocycline and tigecycline and resistance to bacteriocins.

O3-4: Evolution towards increased carbapenemase activity in OXA-51-like beta-lactamases of *Acinetobacter baumannii*

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The OXA-51-like β -lactamases are a large group of enzymes intrinsic to *Acinetobacter baumannii*. In the absence of an acquired carbapenemase, *A. baumannii* can achieve clinical resistance to carbapenems by increasing expression of their OXA-51-like enzyme. However, not all *A. baumannii* are carbapenem-resistant, and there is evidence that different OXA-51-like variants confer different levels of resistance. Furthermore, it is not clear the degree to which OXA-51-like enzymes interact with other mechanisms such as efflux and permeability to confer a resistant phenotype. This study used genomics, proteomics and cloning of *bla*_{OXA-51-like} variants to determine their contribution to carbapenem resistance.

Ten clinical *A. baumannii* strains with no acquired carbapenemase were genome sequenced with Illumina technology. Positions of IS elements were confirmed by PCR. Total proteome analysis of all strains was conducted using LC-MS/MS. 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* DH5 α , *A. baumannii* CIP70.10 and *A. baumannii* BM4547, and the MICs of carbapenems measured. All OXA-51-like sequences present in the NCBI genome sequence database were downloaded and a maximum likelihood phylogeny estimated.

Analyses of genomic and proteomic data did not identify any mechanisms that could explain the carbapenem-resistant phenotype of the strains, with the exception of the OXA-51-like β -lactamases. Only three cloned enzyme variants conferred a greater than 2-fold increase in carbapenem MICs: 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 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. IS elements were more frequently associated with variants thought to confer carbapenem resistance.

Amino acid substitutions surrounding the enzyme active site are responsible for increasing the ability of OXA-51-like variants to hydrolyse carbapenems, conferring reduced phenotypic susceptibility without contributions from other resistance mechanisms. These substitutions have occurred on many separate occasions, representing widespread evolution towards reduced carbapenem susceptibility in *A. baumannii* independent of acquired carbapenemases.

Keynote Lecture 4:

Regulation of a virulence switch in *Acinetobacter baumannii*

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Acinetobacter baumannii AB5075 and many clinical isolates can rapidly switch between two phenotypically distinct subpopulations, distinguished by their opaque or translucent colony phenotypes under oblique lighting. Multiple phenotypic differences exist between opaque, designated VIR-O and translucent (AV-T) variants, including biofilm formation, motility, quorum-sensing signal production, and capsule thickness. The VIR-O variant is highly virulent in a mouse lung model of infection. In contrast, the AV-T variant is rapidly killed *in-vivo* and is highly susceptible to host defenses. The VIR-O to AV-T switch is regulated in part by *ABUW_1645*, encoding a TetR-type regulator, where overexpression drives cells from the VIR-O to AV-T state. Additional TetR-type regulators have now been identified that can also drive VIR-O cells to the AV-T state when overexpressed. This suggests that complex and potentially redundant pathways control VIR-O to AV-T switching. In addition, we have recently determined that VIR-O variants can exhibit highly variable rates of switching to AV-T. "Normal" VIR-O variants switch to AV-T at a rate of ~5-10% in twenty-four old colonies. However, two additional variants were recently identified; one variant designated low-switching VIR-O (LSO) switched to AV-T at a 1,000-fold lower rate. The second variant, hyper-VIR-O, exhibited a 10-fold increased rate of switching compared to normal VIR-O. A mouse lung infection model demonstrated that each variant had markedly different virulence properties than the normal VIR-O strain. PacBio whole genome sequencing between normal VIR-O and the LSO variant revealed differences in the copy number of a region flanked by integrase (*intl*) genes encoded on the large plasmid p1AB5075. The normal VIR-O had two copies and the LSO had one copy. In the hyper-VIR-O, this region was amplified to approximately 20 copies. When this region was cloned on a multicopy plasmid, it was sufficient to restore normal switching from VIR-O to AV-T in the LSO variant. The gene responsible for this phenotype is currently under investigation.

O4-1: Characterisation of antimicrobial resistance and virulence phenotypes in two-component regulatory system mutants from AB5075

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Acinetobacter baumannii is currently considered as one of the top nosocomial pathogens and we desperately need novel drug targets and strategies to challenge it. Two-component systems (TCS) are playing an important role in the regulation of virulence factors. Here we have characterised the antimicrobial susceptibility and virulence potential of putative TCS mutants derived from the model strain AB5075.

The AB5075 has been shown to exhibit a double phase phenotype, with opaque and translucent variants differing in terms of virulence and ability to form biofilm. To account for this phenotype, opaque and translucent variants have been considered as independent entities and compared with the corresponding wild-type variants.

22 knock-out mutants defective in TCS as well as in additional putative transcriptional regulators were acquired from a commercial mutant library (<http://www.gs.washington.edu/labs/manoil/baumannii.htm>). Antimicrobial resistance was studied by gradient diffusion and the virulence potential of all mutants was assessed by measuring biofilm formation and lethality in a *Galleria mellonella* infection model.

The only antibiotics affected were aminoglycosides, with a two-fold reduction of MIC in all mutant strains, except for 2 mutants that showed a higher reduction in their MICs. Regarding biofilm formation, 7 out of the 22 mutant strains presented differences exclusively in the translucent variant, 4 of them showed a decrease in biofilm formation, whereas the other 3 showed an increase. Only the opaque variant of one mutant strain presented a decrease in biofilm formation. Unexpectedly, we observed that both phase variants of 4 mutant strains showed a variation in their biofilm formation ability. In the infection assay model, 7 translucent mutant strains exhibited increased lethality and only one of the opaque variants showed decreased virulence. Interestingly, 2 translucent variants that already showed reduced resistance and biofilm production exhibited increased virulence. Similarly, in those mutant strains with decreased biofilm formation in both phase variants, only the translucent variant presented increased virulence.

This study represents a preliminary screening for TCS and additional transcriptional regulators governing those genes involved in the virulence phenotypes of *A. baumannii*. Additional studies need to characterize such regulators and identify their regulatory network.

O4-2: *Acinetobacter baumannii* phenotypic variation in response to DNA damage is mediated by a 5'UTR recognized in *Bacillus subtilis*

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Acinetobacter baumannii forms two cell types in response to DNA damage. One cell type has low while the other has high expression of DNA damage response (DDR) genes [1]. This phenotypic variation in response to DNA damage serves as a bet-hedging strategy and is dependent on a 5'untranslated region (UTR) in the *recA* gene transcript [2]. We have searched the public databases and find the UTR sequence in all *A. baumannii* strains sequenced to date, suggesting this phenomenon is conserved within the species.

The structure of the *recA* 5' UTR is important for the phenotypic variation of DDR gene expression; non-structured UTR derivatives results in low uniform expression, which is partly due to transcript stability. To determine if the *recA* 5'UTR alone can alter the expression of a non-DDR gene, we fused the UTR to a fluorescent protein gene (*mKate2*) under a tryptophan promoter in *A. baumannii* and showed that there are two cell types independent of DNA damage; low and a high *mKate2* expression [2]. We hypothesized that the 5'UTR might confer phenotypic variation regardless of the bacterial host. To test this hypothesis, we introduced by recombination the native *A. baumannii recA* 5'UTR and its structural derivatives fused to green fluorescent protein (GFP) under a strong constitutive promoter onto the chromosome of the evolutionarily distant *Bacillus subtilis*. We find that GFP is constitutively expressed in *B. subtilis* with low and high expressing GFP cell types. This evidence suggests either a conserved bacterial regulator governing the UTR structure, or that the UTR is intrinsically and stochastically regulated to mediate two levels of gene expression. This work provides evidence for an evolutionarily conserved bet-hedging mechanism through a cis-regulatory element that could help mediate survival in response to stress or environmental changes.

[1] MacGuire AE, Ching MC, Diamond BH, Kazakov A, Novichkov P, Godoy VG. 2014. Activation of Phenotypic Subpopulations in Response to Ciprofloxacin Treatment in *Acinetobacter baumannii*. *Mol. Microbiol.* **92**: p 138-152.

[2] Ching, C., Gozzi, K., Heinemann, B., Chai, Y. & Godoy, V. G. 2017. RNA-Mediated cis Regulation in *Acinetobacter baumannii* Modulates Stress-Induced Phenotypic Variation. *J. Bacteriol.* **199**, 1–15.

O4-3: Interplay between the RND pump AdeIJK and transcriptional regulator AdeN impacts the antibiotic resistance and virulence of *Acinetobacter baumannii*

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Efflux-mediated resistance by proteins belonging to the Resistance-Nodulation-Division (RND) family is the major contributor to intrinsic antibiotic resistance of *A. baumannii*. AdeIJK is a constitutively expressed efflux pump of *A. baumannii* that confers resistance to multiple classes of antibiotics. Expression of AdeIJK is regulated by AdeN, a TetR-family protein, encoded 813kb upstream of *adeIJK* operon. In order to understand the interplay between AdeN and AdeIJK in antibiotic resistance and virulence of *A. baumannii*, we used novel molecular tools designed in our laboratory. The *adeN* gene deletion created in *A. baumannii* ATCC17978 displayed a differential expression of more than 100 genes. In order to determine if this differential expression was a result of the absence of AdeN or overexpression of the AdeIJK, we also created $\Delta adeIJK$ and $\Delta adeN:\Delta adeIJK$ derivatives of *A. baumannii* ATCC17978. Gene complementation was achieved using the single copy gene expression system. Using this system, we were able to regulate the expression of *adeN* without the overexpression of the AdeIJK pump. Conversely, we were also able to control the expression of *adeIJK* independent of AdeN. Antibiotic susceptibility and phenotypes were assessed to understand the impact of *adeN* or *adeIJK* independently. Our findings indicate that the changes in antibiotic susceptibility were dependent on the AdeIJK pump. Interestingly, we observed that virulence of *A. baumannii* in *Galleria mellonella* infection model was dependent on the presence of both AdeN as well as AdeIJK. Our data suggest that AdeIJK pump is not only involved in not only in the antibiotic resistance of *A. baumannii* but also its virulence. However, virulence of *A. baumannii* is also dependent on the presence of AdeN, independent of AdeIJK.

O4-4: Identification of the diversity of *Acinetobacter baumannii* type VI secretion system effectors and characterisation of a novel effector/immunity pair

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The type VI secretion system (T6SS) is a bacterial nanomachine utilised by many Gram-negative bacteria for microbial warfare and/or manipulation of host processes. *Acinetobacter baumannii*, a nosocomial pathogen, utilises the T6SS to deliver antibacterial effector proteins to surrounding bacterial cells. These antibacterial effectors are predicted to be delivered via specific interaction with cognate VgrG proteins, which form part of the tip of the T6SS spike. In this project we aimed to identify the range of T6SS effectors produced by different *A. baumannii* strains. As genes encoding T6SS effectors are often found downstream of *vgrG* genes, we first used a range of bioinformatic techniques to identify the *vgrG* loci in 97 *A. baumannii* complete genomes. Predicted effector and immunity genes were then identified downstream of each *vgrG* gene. Phylogenetic analysis of the predicted effectors revealed that they clustered into 32 distinct groups, with some having predicted amidase, chitinase, lipase, nuclease, or deaminase functional domains. Almost all the identified effectors showed no, or very low, identity to characterised T6SS antibacterial effectors from other species. Two predicted effectors, designated Type six secretion system exported 5 *A. baumannii* (Tse5^{Ab}), and Tse6^{Ab}, were further characterized. Both Tse5^{Ab} and Tse6^{Ab} contain a Rearrangement Hot Spot (RHS) domain that is predicted to fold around a short toxic domain located at the C-terminal end of the same protein. Recombinant expression of the predicted toxic C-terminal domain of Tse5^{Ab} in both the cytoplasm and periplasm (via Sec export) did not perturb *E. coli* growth, but expression of the equivalent region of Tse6^{Ab} in the cytoplasm was toxic to *E. coli*; this toxicity was neutralised by expression of the cognate immunity protein, designated Type six secretion system immunity 6 *A. baumannii* (Tsi6^{Ab}). Analysis of the Tse6^{Ab} amino acid sequence identified a Tox-GHH nuclease domain common to proteins that function as DNases. Site-directed mutagenesis of this GHH motif (to AAA) inactivated the toxic activity of this protein when expressed in *E. coli*. Taken together, these data show that different *A. baumannii* strains express a very diverse set of T6SS antibacterial effectors and it is likely that the precise set of effector/immunity pairs expressed in any strain plays a crucial role in inter-strain competitive interactions. Future characterisation of the diverse set of *A. baumannii* T6SS antibacterial effectors may lead to the identification of antibacterial molecules with novel activities.

Keynote Lecture 5:

Laboratory Evolution Made EASy: Expanding Metabolism in *Acinetobacter baylyi* ADP1

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Gene duplication and divergence are fundamental steps in evolution. In nature, these processes tend to occur rarely and stochastically, resulting in a slow pace of evolutionary change. To accelerate the rate of obtaining bacterial strains with novel and desirable features in the laboratory, a method was devised to exploit the unique genetic system of *Acinetobacter baylyi* ADP1 to create specifically duplicated (and further amplified) regions of the chromosome. This method, called Evolution by Amplification and Synthetic biology (EASy), conferred growth on guaiacol, an aromatic compound not normally degraded by the wild-type strain (ADP1). This trait was acquired by the addition of foreign genes and the use of EASy to select necessary changes in that DNA [1,2]. As described here, the EASy method was developed further and used to increase the funneling of lignin-derived aromatic compounds into central metabolism. Excess carbon can then be diverted to storage compounds, such as wax esters, and further metabolic engineering applied for biosynthetic purposes. The goal of this project is to improve the conversion of processed lignin streams into valuable compounds (lignin valorization) [3]. Current methods to generate bioenergy from renewable lignocellulosic materials are not yet economically feasible. In large part, this problem stems from the lignin portion of biomass being vastly underutilized. Our current studies involve the insertion of large segments of foreign DNA, which encode complicated catabolic pathways, into the *A. baylyi* chromosome. As strains with new metabolic capabilities are selected, whole genome sequencing is being used to detect chromosomal changes. In most cases, multiple mutations are detected. New methods are being developed for the rapid assessment of which mutation(s) are required for phenotypic changes. In some cases, these genetic analyses revealed genes/proteins that play important, and previously unknown, roles in aromatic compound catabolism.

[1] M. Tumen-Velasquez, C. W. Johnson, A. Ahmed, G. Dominick, E. M. Fulk, P. Khanna, S. A. Lee, A. L. Schmidt, J. G. Linger, M. A. Eiteman, G. T. Beckham, E. L. Neidle. Accelerating pathway evolution by increasing the gene dosage of chromosomal segments. *Proc. Natl. Acad. Sci. USA* **115** (2018) 7105–7110.

[2] S. J. B. Mallinson, M. M. Machovina, R. L. Silveira, M. Garcia-Borràs, N. Gallup, C. W. Johnson, M. D. Allen, M. S. Skaf, M. F. Crowley, E. L. Neidle, K. N. Houk, G. T. Beckham, J. L. DuBois, J. E. McGeehan. A promiscuous cytochrome P450 aromatic O-demethylase for lignin bioconversion. *Nat. Comm.* **9** (2018) 2487.

[3] J. G. Linger, D. R. Vardon, M. T. Guarneri, E. M. Karp, G. B. Hunsinger, M. A. Franden, C. W. Johnson, G. Chupka, T. J. Strathmann, P. T. Pienkos, G. T. Beckham. Lignin valorization through integrated biological funneling and chemical catalysis. *Proc. Natl. Acad. Sci. USA*. **111** (2014) 12013-12018.

O5-1: Metabolic engineering of *Acinetobacter baylyi* ADP1 for upgrading non-conventional substrates

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Although biological production often provides a more sustainable alternative for chemical synthesis of various products, it is essential to consider the socio-economic impacts of the bioprocess. Using an abundant and cheap substrate that is preferably considered waste (such as lignin and short organic acids), has a great significance on the feasibility and sustainability of the process. Alas, the most commonly used production hosts, such as *Escherichia coli* and *Saccharomyces cerevisiae*, cannot efficiently utilize or tolerate some of these broadly abundant carbon sources. As a potential host for upgrading challenging substrates, we have established tools and engineering strategies for the production of a variety of industrially relevant products, namely wax esters, 1-alkenes and alkanes, from acetate and lignin derived molecules using *Acinetobacter baylyi* ADP1. In addition, we have investigated the strain's capabilities in lignin depolymerization and lignin monomer tolerance and utilization. Our studies promote the use of *A. baylyi* ADP1 as a potential chassis for the valorization or recalcitrant substrates.

O5-2: A regulator discovered to enhance lignin valorization by *Acinetobacter baylyi* ADP1 affects switching between virulent and avirulent states of *Acinetobacter baumannii* AB5075

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Acinetobacter studies often focus either on metabolism of environmental isolates, such as *A. baylyi* ADP1, or on virulence of pathogenic strains, such as *A. baumannii* AB5075. In our studies, CsrA (also known as RsmA) was found to be important in different contexts. Using a novel lab evolution method [1], gain-of-function *csrA* alleles arose in efforts to engineer ADP1 to convert lignin-derived aromatics to valuable compounds, a process termed lignin valorization. Renewed interest in lignin degradation stems from applications in bioenergy and biotechnology. Experiments and computational predictions indicated that CsrA acts as a positive post-transcriptional global regulator of aromatic compound catabolism, a role not previously attributed to CsrA.

In other bacteria, CsrA affects virulence, thereby raising questions about its function in pathogenic *Acinetobacter*s. Functionality was tested in *A. baumannii* AB5075 using a transposon-disrupted *csrA* mutant [2]. This mutant had a significantly altered frequency of switching between two phenotypes [3]: virulent opaque (VIR-O) and avirulent translucent (AV-T) colonies. The wild-type switching frequency was restored by *csrA* complementation.

Studies in *A. baylyi* ADP1 and *A. baumannii* AB5075 continue to characterize the molecular basis of both CsrA-mediated regulation of aromatic compound metabolism and CsrA-mediated influence on virulence state switching. Pathogenicity studies are being done with the waxworm *Galleria mellonella*.

[1] Tumen-Velasquez M, Johnson CW, Ahmed A, *et al.* (2018) Accelerating Pathway Evolution by Increasing the Gene Dosage of Chromosomal Segments. *Proc Natl Acad Sci USA* **115**:7105-7110.

[2] Gallagher LA, Ramage E, Weiss EJ, *et al.* (2015) Resources for Genetic and Genomic Analysis of Emerging Pathogen *Acinetobacter baumannii*. *J Bacteriol* **197**:2027–2035.

[3] Chin CY, Tipton KA, Farokhyfar M, Burd EM, Weiss DS, Rather PN. (2018) A high-frequency phenotypic switch links bacterial virulence and environmental survival in *Acinetobacter baumannii*. *Nat Microbiol* **3**:563–569.

O5-3: Molecular regulation of *Acinetobacter baumannii* biofilm formation

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Acinetobacter baumannii is a formidable opportunistic pathogen, the persistence of which can, at least in part, be attributed to its biofilm forming capacity. For clinical isolates, a strong correlation between antibiotic resistance, virulence, and the ability to adhere to clinically relevant surfaces is evident. Despite this, little is known regarding the mechanisms which govern biofilm formation in *A. baumannii*. Herein, we present our findings on the transcriptional regulation of biofilm formation for the multidrug resistant osteomyelitis isolate, AB5075. Using xCELLigence, a newly developed real-time tracking technology, we measured biofilm adherence, progression, and dispersal. RNA-seq based transcriptomic profiling of adhered cell populations compared to their planktonically grown counterparts revealed 142 genes upregulated ≥ 3 -fold within the biofilm including cold shock protein *csp1*, heat shock protein *hsIR*, and additional transcriptional regulators (*mprA*, *badM* and *rhaS*). Using an *A. baumannii* transposon library in conjunction with classical biofilm assays, we show that disruption of these factors resulted in decreased biofilm production. Further to this, we observed increased susceptibility to protein synthesis inhibiting antibiotics and ethidium bromide stress for the *csp1*, *hsIR*, and *recX* mutants. Ethidium bromide efflux assays demonstrate this increased susceptibility may be due to diminished efflux capacity of *recX* and *hsIR* mutants in addition to impaired biofilm formation. Regarding the *csp1* mutant, cold shock proteins have been previously implicated in transcriptional responses to both antibiotic stress and nutrient limited conditions – a common characteristic of a biofilm. Further investigations of Csp1 revealed a nucleic acid binding domain in the form of an RNP-1 motif, a feature conserved among many cold shock proteins. Current work is ongoing to test Csp1's DNA/RNA binding ability in order to identify molecular targets through which it may influence biofilm formation. Collectively, our findings reveal a cadre of new biofilm associated genes within *A. baumannii* and provide insight into the global regulatory network of this emerging human pathogen.

O5-4: Adaptation of *Acinetobacter baumannii* to the human host and to osmotic stress

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In the last decades *Acinetobacter baumannii* is an increasing threat in hospitals worldwide, due to the rising number of infections with multi- or even pan-resistant strains. However, no typical virulence factors, such as exotoxins are known, indicating that *A. baumannii* is well-adapted to the human host. A unique trait of *A. baumannii* is to persist on dry surfaces or under hyperosmotic conditions over long periods, which suggests extraordinary evolved osmotic stress protection mechanisms. One important factor in osmotic stress protection in bacteria is the uptake of compatible solutes via members of the betaine/choline/carnitine (BCC) transporter family, but not much is known about this transporter class in *A. baumannii*. In the genome of the human pathogen six potential BCC-type transporter genes are annotated, *betT1*, *betT2*, *betT3*, *betTY*, *betTZ* and *aci01347*. Three of them are known from the soil bacterium *Acinetobacter baylyi*. Two have already been identified as choline transporters, one osmotic stress dependent (BetT2) and one independent (BetT1) [1] and one BCC-type transporter has been identified to mediate osmo-dependent betaine uptake (BetT3) [2]. However, nothing is known about BetTY, BetTZ and Aci01347.

To get insights into the substrate specificity and the osmotic stress dependence, we heterologously expressed these three transporters in *Escherichia coli* MKH13, which lacks compatible solute transporters and performed uptake studies with radioactively labelled substrates in the presence of different osmolalities. These studies revealed, that *A. baumannii* has two further betaine transporters, one osmotic stress independent (BetTZ) and one osmotically activated (BetTY) and one further osmotic stress independent choline transporter (Aci01347). We could also show that Aci01347 is not only responsible for choline uptake, but also accumulates carnitine in a salt stress independent fashion [3]. The latter is quite unique, due to the fact, that all known carnitine transporters of this family are substrate:product antiporter and there is no experimental evidence, that Aci01347 exports γ -butyrobetaine during the uptake of carnitine.

The uptake of carnitine is a clear metabolic benefit of *A. baumannii* due to its ability to grow with carnitine as sole carbon and energy source. To elucidate the role of Aci01347 in uptake of carnitine as carbon source we performed mutant studies which provide clear evidence, that this transporter is the only carnitine transporter in *A. baumannii*.

The high abundance of carnitine in the human host and the presence of further osmotic stress independent BCC-type transporters suggest a role of these transporters in adaptation of *A. baumannii* to the human host.

[1] M. Sand, J. Stahl, I. Waclawska, C. Ziegler and B. Averhoff. Identification of an osmo-dependent and osmo-independent choline transporter in *Acinetobacter baylyi*: implications in osmotic stress protection and metabolic adaptation. *Environ. Microbiol.* **16** (2014), 1490-1502.

[2] M. Sand, V. De Barardinis, A. Mingote, H. Santos, S. Göttig, V. Müller and B. Averhoff. Salt adaptation in *Acinetobacter baylyi*: identification and characterization of a secondary glycine betaine transporter. *Arch. Microbiol.* **193** (2011), 723-730.

[3] J. Breisch, I. Waclawska and B. Averhoff. Identification and characterization of a carnitine transporter in *Acinetobacter baumannii*. *Microbiologyopen.* e752 (2018).

Keynote Lecture 6:

Dry and salty: how *Acinetobacter* copes with low water stress

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Desiccation and addition of salt have the same physicochemical consequence for a cell: 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 [1]. To study the molecular basis of desiccation resistance in *Acinetobacter baumannii* [2], we address the nature and role of compatible solutes in *A. baumannii* as well as their role in infection.

As a response to osmotic stress, *A. baumannii* accumulates glycine betaine (uptake) and glutamate (synthesis) and the rather unusual solute mannitol (synthesis) as well as trehalose (synthesis) in minor amounts [3,4]. I will describe the genes involved in synthesis and their regulation, as far as it is known to date. One of the most important questions is how low water activity is measured and the intracellular potassium concentration may be used as a signal. Therefore, we studied K⁺ uptake in wild type and in transporter mutants.

Together with genome wide expression profiling, our studies culminate in a first comprehensive overview of the cellular and molecular response of *A. baumannii* to low water activities in its environments [5].

[1] Roeßler, M., and Müller, V. (2001) Osmoadaptation in bacteria and archaea: common principles and differences. *Environ Microbiol* **3**: 743-754.

[2] Zeidler, S., and Müller, V. (2019) The role of compatible solutes in desiccation resistance of *Acinetobacter baumannii*. *MicrobiologyOpen* **8**: e740.

[3] Zeidler, S., Hubloher, J., Schabacker, K., Lamosa, P., Santos, H., and Müller, V. (2017) Trehalose, a temperature- and salt-induced solute with implications in pathobiology of *Acinetobacter baumannii*. *Environ Microbiol* **19**: 5088-5099.

[4] Zeidler, S., Hubloher, J., König, P., Ngu, N.D., Scholz, A., Averhoff, B., and Müller, V. (2018) Salt induction and activation of MtID, the key enzyme in the synthesis of the compatible solute mannitol in *Acinetobacter baumannii*. *MicrobiologyOpen* **7**: e614.

[5] Zeidler, S., and Müller, V. (2019) Coping with low water activities and osmotic stress in *Acinetobacter baumannii*: significance, current status and perspectives. *Environ Microbiol*: **21**: 2212-2230.

O6-1: Structure of the osmotic stress protein MtlD from *A. baumannii*

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One of the main reasons for the persistence of *Acinetobacter baumannii* and recurrent outbreak in clinical settings is its remarkable ability to tolerate and survive under extreme desiccation stress. The synthesis of mannitol as compatible solute to overcome the effects of hyperosmolarity in *A. baumannii* is catalyzed by MtlD, a novel bifunctional mannitol-1-phosphate dehydrogenase/phosphatase [1]. This enzyme catalyzes in a first step the reduction of fructose-6-phosphate to mannitol-1-phosphate with NADPH as a cofactor, followed by the dephosphorylation of mannitol-1-phosphate to mannitol [2]. Systematic screening for protein stability and crystallization trials of MtlD has led to the crystal structure of unliganded MtlD at 2.5 Å in resolution. MtlD is organized as an elongated antiparallel dimer. The dehydrogenase and phosphatase active sites are oppositely located at the distal ends of each protomer. This observation might indicate that MtlD is active as a dimer, as the dehydrogenase domain of one protomer is adjacent to the phosphatase domain of the other protomer. Interestingly, a long helix-loop-helix structure extending from each protomer links the respective dehydrogenase domain to the phosphatase domain of the neighbouring subunit. We speculate that this feature is central to conformational changes leading to the coupled dehydrogenase/phosphatase catalysis.

[1] S. Zeidler, J. Hubloher, P. König, N. D. Ngu, A. Scholz, B. Averhoff and V. Müller. Salt induction and activation of MtlD, the key enzyme in the synthesis of the compatible solute mannitol in *Acinetobacter baumannii*. *Microbiologyopen* **7** (2018), e00614.

[2] M. Sand, A. I. Mingote, H. Santos, V. Müller and B. Averhoff. Mannitol, a compatible solute synthesized by *Acinetobacter baylyi* in a two-step pathway including a salt-induced and salt-dependent mannitol-1-phosphate dehydrogenase. *Environ. Microbiol.* **15** (2013), 2187-2197.

O6-2: Molecular basis for desiccation tolerance in *Acinetobacter baumannii*

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A key factor making *A. baumannii* a notorious nosocomial pathogen is thought to be its exceptionally high desiccation tolerance. To understand the functional basis of this trait, we used transposon-sequencing (Tn-seq) to identify genes contributing to desiccation tolerance in *A. baumannii*, strain AB5075. We identified 142 candidate desiccation tolerance genes, five of which were verified to be particularly significant by single mutant analysis. One of these, the global post-transcriptional regulator gene *csrA*, was characterized in more detail using proteomic analysis to identify genes it regulates. Two of the regulated genes, both encoding proteins of unknown function, showed severe mutant defects in desiccation tolerance. Desiccation tolerance was largely restored when each mutant was assayed in the absence of oxygen, indicating that they may protect bacteria from oxidative damage during desiccation. A *csrA* deletion mutant remained sensitive to desiccation even in the absence of oxygen, suggesting that CsrA controls the translation of additional genes important for desiccation tolerance.

O6-3: Molecular insights into the mechanisms that define the lipidomic landscape of *Acinetobacter baumannii* under membrane stress

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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 broad-spectrum antibacterial potential. Of these, the omega-6 fatty acid arachidonic acid (AA) and the omega-3 fatty acid docosahexaenoic acid (DHA) are highly abundant, hence, we investigated their effects on *Acinetobacter baumannii*. Our analyses reveal that AA and DHA readily incorporate into the *A. baumannii* membrane and impact membrane integrity. Importantly, our analyses also reveal a role for environmental fatty acids in antibiotic susceptibility and the development of antibiotic resistance in *A. baumannii*. Through transcriptional profiling and mutant analyses, we identified multiple lipid homeostasis mechanisms that play a role in AA and DHA resistance, including the β -oxidation pathway, the AdeIJK RND efflux system and a DesB-like desaturase. This is the first study to examine the antimicrobial effects of host fatty acids on *A. baumannii*, and highlights the potential of AA and DHA to protect against *A. baumannii* infections. Further, the novel role for fatty acids in antimicrobial resistance provides insights into the complex interplay between host factors and anti-*A. baumannii* therapy.

O6-4: Natural transformation promotes horizontal transfer of large antibiotic resistance islands in *Acinetobacter baumannii*

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Causative agent of infections in humans and animals, *Acinetobacter baumannii* concerns because of its startling ability to acquire rapidly antibiotic resistance. Horizontal gene transfer mechanisms, including natural transformation, may explain the high propensity of *A. baumannii* to acquire antibiotic resistance. Contrasting with mechanisms where bacterial cells are passive targets (phage, plasmid, ICE...), this mechanism is used by bacteria to actively seek new genetic material. By allowing uptake and chromosomal integration of exogenous DNA fragments, transformation promotes genetic diversification. Natural transformation, described recently in *A. baumannii* [1] is a conserved trait among clinical and non-clinical isolates of human and animal origin [2]. We here demonstrate that natural transformation occurs in mixed populations of isolates in the absence of exogenously added DNA. We found that co-culture of isolates stimulates DNA release in the medium, allowing both intra- and interspecific genetic transfer at high frequencies (10^4). Horizontal transfer occurs as early as three hours after two isolates are mixed. It is inhibited by DNase I and undetectable in recipient strain deleted of the *comEC* gene, demonstrating the exclusive role of natural transformation. Genome sequencing of transformants confirmed effective transfer, and analysis of SNPs between input strains and transformants delineated recombination tracts, which in some transformants exceeded 130 kb. Importantly, multiple recombination tracts were observed far away from the selected events. Thus, extensive transfer occurs in mixed populations of *A. baumannii* isolates. Notably, these extensive genetic exchange allow horizontal transfer of AbaR islands, the highly diverse genomic islands carrying multiple putative antibiotic-resistance genes but lacking self-mobilizing gene. We show that acquisition of AbaR islands effectively confers resistance to antibiotics. Furthermore, we highlight that natural transformation allows intra- and interspecific acquisition of carbapenem resistance, including when carried by an AbaR genomic island. Hence, *A. baumannii* and related species can efficiently acquire antibiotic resistance and large resistance genomic islands through natural transformation.

[1] G. Wilharm, J. Piesker, M. Laue and E. Skiebe (2013). *Journal of Bacteriology*, **195**, 4146–4153.

[2] A.-S. Godeux, A. Lupo, M. Haenni, S. Guette-Marquet, G. Wilharm, M.-H. Laaberki and X. Charpentier (2018), *Journal of Bacteriology* **200**, e00181-18.

Keynote Lecture 7:

Molecular epidemiology of clinical, animal, and environmental isolates of *A. baumannii*

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It is now recognised that *Acinetobacter baumannii* is not only found in the human-hospital environment, but is also isolated from companion, domestic, and wild animals, as well as from the soil. Furthermore, while the majority of *A. baumannii* associated with humans are isolated mostly from hospitalised patients, *A. baumannii* can also be community acquired. Therefore we have at least 6 different ecosystems where this organism can be found. The use of multilocus sequence typing has given us a good understanding on the relationship between strains isolated from these different ecosystems, and with the recent advent of relatively cheap whole genome sequencing, has allowed us a greater resolution in bacterial typing and understanding of their populations.

To date, we have identified at least nine international clonal lineages (IC1-9) that have been isolated from hospitalised patients in multiple countries and continents. Often multidrug, and sometimes pan-drug resistant, these are features that are rarely seen in the other ecosystems, reflecting the antibiotic pressure in the hospital.

Although sharing a propensity for antimicrobial resistance, there are some significant differences between the ICs. For example, IC2 is by far the most commonly found lineage in the hospital, isolated on all continents with the exception of South America where the prevalent lineage is IC5. Sequence type 10 (IC8) has recently been associated with community acquired *A. baumannii* in Australia.

In contrast, *A. baumannii* isolated from cattle, wild animals, and soil, are most often unique and show little, or no clonality, although IC7 and IC8-like strains have been isolated from avian sources, whereas those from veterinary hospitals are closer to those associated with human hospitals. Therefore while we have identified 6 ecosystems where *A. baumannii* can be isolated, there are in fact two major *A. baumannii* ecosystems with their own special features and with few overlaps; (i) hospital associated which exhibit antibiotic resistance and clonality, and (ii) the wider environment made up of antibiotic susceptible and epidemiologically unique isolates. This presentation will discuss these features of *A. baumannii*, and identify areas where there are gaps in our knowledge.

07-1: *Acinetobacter baumannii* – jet stream rider

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The natural reservoirs of the nosocomial pathogen *Acinetobacter baumannii* are poorly defined. In previous work we identified white storks as a model system to study the ecology of *A. baumannii* [1]. Having screened more than 1,200 white stork nestlings over a period of six years in different regions of Poland and Germany (overall isolation rate ~25%) and having included food chain analyses and environmental samplings, we come up with a detailed picture of the dynamics and diversity of *A. baumannii* in natural habitats. Adult storks, rather than being stably colonized with *A. baumannii* strains that are then transferred to their offspring, come in contact with *A. baumannii* while foraging. Among their prevailing food sources consisting of earthworms, mice, and insects, we identified earthworms as a potential source of *A. baumannii*, but also the associated soil as well as plant roots. Concrete soil and compost habitats were identified that allow population dynamics to be studied over the course of the year. The prevalence of *A. baumannii* exhibited a strong seasonality and peaked in summer. Decomposition of plant material under fungal regime appears to set the stage for proliferation of *A. baumannii*. The diversity found reached up to 10 distinct lineages isolated from a single sample site on a single day. Extensive horizontal gene transfer was detected between co-colonizing lineages. Over a season, more than 20 distinct lineages could be isolated from individual sample sites. The strains we collected in Poland and Germany represent more than 50% of the worldwide known diversity in terms of the intrinsic OXA-51-like β -lactamase. Core-genome based phylogenetic analyses illustrate numerous linkages between wildlife isolates and hospital strains, with the respective last common ancestors dating back hundreds to thousands of years. Our data suggest a persistent worldwide spread of *A. baumannii* lineages since ancient times. Linking published work on the interaction between *A. baumannii* and fungi [2-5], on aspergillosis as a major cause of mortality in white stork nestlings [6], and our findings, we hypothesized that fungi and *A. baumannii* share a long history of coevolution. Interaction studies revealed the capability of *A. baumannii* to adhere to fungal spores and suppress spore germination. Taken together, the intrinsic resistance endowment and potential to acquire antibiotic resistance can be explained from coevolution with antibiotics-producing fungi, and resistance to desiccation stress and radiation can be interpreted in the light of intercontinental hitchhiking on fungal spores.

[1] G. Wilharm, E. Skiebe, P.G. Higgins et al. Relatedness of wildlife and livestock avian isolates of the nosocomial pathogen *Acinetobacter baumannii* to lineages spread in hospitals worldwide. *Environ. Microbiol.* **19** (2017), 4349-4364.

[2] M.G. Smith, S.G. Des Etages, M. Snyder. Microbial synergy via an ethanol-triggered pathway. *Mol. Cell. Biol.* **24** (2004), 3874-3884.

[3] J.A. Gaddy, A.P. Tomaras, L.A. Actis. The *Acinetobacter baumannii* 19606 OmpA protein plays a role in biofilm formation on abiotic surfaces and in the interaction of this pathogen with eukaryotic cells. *Infect. Immun.* **77** (2009), 3150-3160.

[4] X. Kostoulas, G.L. Murray, G.M. Cerqueira et al. Impact of a cross-kingdom signaling molecule of *Candida albicans* on *Acinetobacter baumannii* physiology. *Antimicrob. Agents Chemother.* **60** (2015), 161-167.

[5] X. Tan, S. Xhu, D. Yan et al. *Candida* spp. airway colonization: A potential risk factor for *Acinetobacter baumannii* ventilator-associated pneumonia. *Med. Mycol.* **54** (2016), 557-566.

[6] P. Olias, A.D. Gruber, B. Winfried, H.M. Hafez, M. Lierz. Fungal pneumonia as a major cause of mortality in white stork (*Ciconia ciconia*) chicks. *Avian Dis.* **54** (2010), 94-98.

O7-2: Resistome and genomic epidemiology of *Acinetobacter spp.* and *A. baumannii* isolated from the natural non-hospital environment

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The rise of antimicrobial resistance (AMR) is now recognised as a global medical crisis, with carbapenem-resistance being found in 62% of *Acinetobacter baumannii* clinical isolates in Singapore [1]. These studies were limited to hospital environment and patients [2]. We hypothesised that AMR may originate from the environmental microbiome, and evolved through either vertical or horizontal gene transfer to generate diversity. We aim to study the resistome and genomic epidemiology of *Acinetobacter spp.* in the natural non-hospital environment locally and regionally. *Acinetobacter spp.* (n = 157) were isolated from soil and water samples collected from East and South Asia including Singapore, Philippines, Malaysia and Indonesia, using selective *Acinetobacter spp.* medium. Species identification was confirmed using MALDI-TOF-MS. Subsequently, antibiotics susceptibility testing and whole genome sequencing were performed. We have characterized *A. baumannii* that were uniquely resistant to carbapenems and co-trimoxazole, as well as *Acinetobacter spp.* that were resistant to tetracycline (*tet* gene) and co-trimoxazole (*sul* gene). We have found carbapenem resistance in *A. baumannii* mediated by a novel 7.8 kb *bla*OXA-72 (*bla*OXA-24-like) harbouring plasmid and co-trimoxazole resistance through *sul* resistance gene in another *A. baumannii* isolate. This isolate shared the same sequence type (ST) as a known clinical isolate. These suggest that the resistance genes are already circulating in the natural environment, and may be horizontally transferred within natural bacterial populations. Currently, there is no local report of community-acquired infections from the environment yet. Antibiotic selection pressure may be a driving force for drug resistance in the clinical setting. Our preliminary study highlights the importance of characterising the natural environmental reservoirs of *A. baumannii* and *Acinetobacter spp.* to understand the origins of resistome.

[1] Hsu, L.Y., Apisarnthanarak, A., Khan, E., Suwantararat, N., Ghafur, A., and Tambyah, P.A. (2017). Carbapenem-Resistant *Acinetobacter baumannii* and *Enterobacteriaceae* in South and Southeast Asia. *Clin Microbiol Rev* 30, 1-22.

[2] Cai, Y., I. Venkatachalam, N. W. Tee, T. Y. Tan, A. Kurup, S. Y. Wong, C. Y. Low, Y. Wang, W. Lee, Y. X. Liew, B. Ang, D. C. Lye, A. Chow, M. L. Ling, H. M. Oh, C. A. Cuvin, S. T. Ooi, S. K. Pada, C. H. Lim, J. W. C. Tan, K. L. Chew, V. H. Nguyen, D. A. Fisher, H. Goossens, A. L. Kwa, P. A. Tambyah, L. Y. Hsu & K. Marimuthu (2017) Prevalence of Healthcare-Associated Infections and Antimicrobial Use Among Adult Inpatients in Singapore Acute-Care Hospitals: Results From the First National Point Prevalence Survey. *Clin Infect Dis*, 64, S61-S67.

O7-3: Poly-clonal dissemination of *Acinetobacter baumannii* in Egyptian Hospitals

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Acinetobacter baumannii is listed as one of the 'priority pathogens' by the World Health Organization due to its multi-drug resistance and global spread. *A. baumannii*, however, is poorly investigated in many low- and middle-income countries, thereby creating data gaps on the epidemiology of this pathogen. The data presented here is a multi-centre study of the molecular epidemiology of *A. baumannii* in Egypt.

167 *A. baumannii* samples were collected from 2010-2016, from four different hospitals across Egypt. Species was confirmed by the *gyrB* multiplex method [1], and also by sequencing of the intrinsic *bla*_{OXA-51-like} gene [2]. Isolates were typed by the Oxford Multi-locus Sequence Typing (MLST) scheme to assign sequence types (ST), clonal complexes (CC), and associated to International clones (IC). Susceptibility testing was performed according to the BSAC guidelines, and the acquired carbapenemases OXA-23, -24/40, -58, NDM, IMP, and VIM were amplified and sequenced.

Our findings indicated a heterogeneous population of 45 different STs, 15 of which were novel. IC-2 was the predominant clone, which is concurrent with global data. STs associated with IC-1, -4, -5 and -8 were also identified, in addition to singletons. OXA-51-like typing further confirmed the diversity, with 19 different variants detected, including the novel OXA-510 and OXA-511. Carbapenem resistance was detected in 93% of isolates, and predominantly associated with OXA-23, but also OXA-40, OXA-58, NDM-1, -2 and VIM-1.

A. baumannii is a problematic global pathogen, and requires accurate epidemiological investigation. Our results indicate the presence of a large pool of epidemiologically diverse MDR *A. baumannii* isolates. IC2-OXA23 is the predominant clone in all hospitals, in addition to the dissemination of other successful clones. This diversity was reflected across all the hospitals, where 18 different STs belonging to different ICs were found in a single hospital. This suggests the continuous introduction of multiple epidemiologically diverse isolates from unknown sources into the hospitals, possibly from environmental and/or zoonotic sources.

[1] P. G. Higgins, M. Lehmann, H. Wisplinghoff, and H. Seifert. *gyrB* multiplex PCR to differentiate between *Acinetobacter calcoaceticus* and *Acinetobacter genomic species 3*. *J. Clin. Microbiol.* **48** (2010) 4592–4594.

[2] S. Pournaras *et al.* Single-locus-sequence-based typing of *bla*_{OXA-51-like} genes for rapid assignment of *Acinetobacter baumannii* clinical isolates to international clonal lineages. *J. Clin. Microbiol.* **52** (2014) 1653–1657.

O7-4: Antibiotic resistance features and molecular epidemiology of *Acinetobacter baumannii* clinical isolates from Pakistan

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Acinetobacter baumannii has emerged as an important opportunistic pathogen worldwide [1]. Our study aimed at investigating the antibiotic resistance features and molecular epidemiology of 52 clinical isolates of *A. baumannii* collected in Pakistan between 2013 (16 isolates from Lahore) and 2015 (36 isolates from Peshawar). Antimicrobial susceptibility patterns were determined by the agar disc diffusion method. Comparative sequence analysis of the *ampC* and *bla*_{OXA-51-like} alleles was used to assign the isolates into clusters. Twenty-five isolates were selected for whole genome sequencing using the MiSeq Reagent Kit v3 and *MiSeq Desktop Sequencer*. MLST and ResFinder, free online applications, were used to determine the isolates' sequence types (ST) and detect the occurrence of acquired antibiotic resistance genes, respectively.

The isolates were divided into 7 clusters and 3 sporadic isolates. Two of the clusters caused outbreaks of infection both in Lahore and Peshawar, two geographically far-off cities. The largest cluster, Ab-Pak-cluster-1, was characterized by *bla*_{OXA-66} and *ISAbal-ampC-19*. It included 24 isolates, belonged to ST2, and was equipped with *bla*_{OXA-23}, *bla*_{PER-1} and several aminoglycoside resistance genes. Seven isolates belonged to cluster-2 (*bla*_{OXA-66}, *ISAbal-ampC-2*, ST2), 4 to cluster-3 (*bla*_{OXA-66}, *ISAbal-ampC-20*, ST2), 3 to cluster-4 (*bla*_{OXA-69}, *ampC-1*, ST1), 2 to cluster-5 (*bla*_{OXA-69}, *ISAbal-ampC-78*, ST1), 4 divided equally between sub-clusters-6A (*bla*_{OXA-371}, *ISAbal-ampC-3*, ST1) and -6B (*bla*_{OXA-371}, *ISAbal-ampC-8*, ST1106), and 5 to cluster-7 (*bla*_{OXA-65}, *ampC-43*, ST158). Each of isolates Lah-01 (*ISAbal-bla*_{OXA-66}, *ISAbal-ampC-2*, ST2), Pesh-04 (*bla*_{OXA-64}, *ampC-25*, ST25) and Pesh-22 (*bla*_{OXA-68}, *ISAbal-ampC-79*, ST23) caused a sporadic infection. Pesh-22, the ST23 isolate, carried the *tet(39)* tetracycline resistance gene. The occurrence of *bla*_{OXA-23}- and *bla*_{GES-11}-positive carbapenem-resistant isolates belonging to ST158 in Pakistan highlighted the clinical concern of this clone and came in line with previous studies suggesting a likely reservoir in the Middle East [2, 3]

Overall, our study detected 7 clusters of *A. baumannii* responsible for local or inter-city outbreaks of hospital-acquired infections, ranging in size between 2 and 24 cases, in Pakistan. The study also demonstrated a valuable application of single- or bi-loci sequence typing schemes for rapid grouping of *A. baumannii* clinical isolates.

[1] L. Dijkshoorn, A. Nemec and H. Seifert. An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*. *Nat. Rev. Microbiol.* **5** (2007), 939-951.

[2] R.A. Bonnin, V.O. Rotimi, M. Al Hubail, E. Gasiorowski E, N. Al Sweih, P. Nordmann, and L. Poirel. Wide dissemination of GES-type carbapenemases in *Acinetobacter baumannii* isolates in Kuwait. *Antimicrob. Agents Chemother.* **57** (2013), 183-188.

[3] A.M. Hammerum, F. Hansen, M.N. Skov, M. Stegger, P.S. Andersen, A. Holm, L. Jakobsen, and U.S. Justesen. Investigation of a possible outbreak of carbapenem-resistant *Acinetobacter baumannii* in Odense, Denmark using PFGE, MLST and whole-genome-based SNPs. *J. Antimicrob. Chemother.* **70** (2015), 1965–1968.

Keynote Lecture 8:

Future alternatives to treat infections caused by *Acinetobacter baumannii*

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Acinetobacter baumannii is amongst the microorganisms in which increased resistance has been observed. However, this situation is not reflected in the development of new antibacterial agents against these bacteria. Recently, WHO has reported a list of antibiotic resistant bacteria to guide the investigation, discovery and development of new antibiotics, mentioning *A. baumannii* in the priority group # 1 (critical). In many cases, the choice of treatment for *A. baumannii* infections is reduced to a single drug, such as colistin, with the risk involved in the development of resistant strains associated with the selective pressure exerted by the use of the antibiotic, having already described panresistant strains of *A. baumannii*. At present, new antibiotics such as ceftazidime/avibactam or ceftolozane, although they may be active against certain multiresistant enterobacteria or *Pseudomonas aeruginosa*, are not active against *A. baumannii*. Therefore, it is evident the need for new antibacterial agents against multi-, extended- and pan-resistant *A. baumannii* strains. Currently, two potential new antibiotics, such as cefiderocol with MIC₉₀ between 0.5 and 8 mg/L and eravacycline with MIC₉₀ 1 mg/L have been tested against *A. baumannii*. Cefiderocol has a potentiality to be used to treat infections caused by these microorganisms although we have to wait for data from the clinical trials in order to evaluate it. However, other potential future alternatives can be considered, among these: 1. Peptides with activity against multidrug-resistant *A. baumannii* with MIC₅₀ of 0.25 mg/L and MIC₉₀ of 0.5 mg/L; 2. Peptidomimetics (ceragenins) with also a good *in vitro* activity with MIC₅₀ and MIC₉₀ of 2 mg/L; 3. Drugs against virulence factors, in this sense a plethora of different cyclic peptides has been studied in regards to the inhibition of OmpA, a virulence factor involved in the adherence of *A. baumannii* to the epithelial cells. Therefore, preventing the development of infection *in vitro* and in a murine sepsis peritoneal model. In conclusion, we should be hopeful in having new antibiotics to treat infections caused by multiresistant strains of *A. baumannii*, both by the antibiotics that are in the pipeline and by the new potential antibacterial agents.

O8-1: Elucidating Mechanisms of Colistin Resistance and the Efficacy of Combination Therapy in MDR/XDR/PDR *Acinetobacter baumannii*

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The rapid dissemination of carbapenem resistance among Gram-negative bacteria, particularly in *Acinetobacter baumannii*, has gained focus on the “old” drug colistin. Nevertheless, the emergence of colistin resistance, last line of defense, causes a serious global threat. Therefore, antimicrobial treatment for these infections remains an important challenge. In this study, we aimed to understand the underlying mechanisms of resistance to colistin and implement a suitable combination therapy. In addition, we aimed at understanding the involvement of the Two-Component system PmrAB and lipid biosynthesis genes in colistin resistance and their corresponding relative expression levels. Interestingly, we noted an increase in the presence of these genes along with decreased levels of resistance and significant over-expression of *pmrAB* genes in colistin resistant isolates [1,2]. We also examined the efficacy of colistin in combination with tigecycline, fosfomycin, zerbaxa, tazocin, teicoplanin, amikacin, levofloxacin, rifampicin and meropenem against colistin resistant *A. baumannii* [3] acquiring various resistance mechanisms. Checkerboard and Time-kill assays demonstrated a synergistic effect with colistin for rifampicin and teicoplanin with bactericidal activity occurring prior to 4 hours of incubation. A preliminary neutropenic pneumonia-induced mouse model, demonstrated improved survival and pathogen clearance from the blood and lung tissue cultures in the rifampicin combination. Up-regulation of *pmr* genes was recorded with a down-regulation of the *lpx* genes *in vitro*, nonetheless this was not the case in *in vivo*. The effect of colistin resistance and the action of colistin was visualized under Scanning Electron Microscopy. In conclusion, understanding the various molecular mechanisms of colistin resistance in *A. baumannii* aided us to correlate the results with combination therapy paving the path for potential treatment options against XDR and PDR infections.

[1] Chopra, Sidharth, Kevin Ramkissoo, and D. C. Anderson. 2013. “A Systematic Quantitative Proteomic Examination of Multidrug Resistance in *Acinetobacter Baumannii*.” *Journal of Proteomics* 84 (June): 17–39. <https://doi.org/10.1016/j.jprot.2013.03.008>.

[2] Fan, Bing, Jie Guan, Xiumei Wang, and Yulong Cong. 2016. “Activity of Colistin in Combination with Meropenem, Tigecycline, Fosfomycin, Fusidic Acid, Rifampin or Sulbactam against Extensively Drug-Resistant *Acinetobacter Baumannii* in a Murine Thigh-Infection Model.” *PLOS ONE* 11 (6): e0157757. <https://doi.org/10.1371/journal.pone.0157757>.

[3] Higgins Paul G, Lehmann M, Wisplinghoff H, and Seifert H. 2010. “*gyrB* multiplex PCR to differentiate between *Acinetobacter calcoaceticus* and *Acinetobacter* genomic species 3.” *J Clin Microbiol* 48(12):4592-4. doi: 10.1128/JCM.01765-10. Epub 2010 Sep 29.

O8-2: A genetic strategy to identify promising *Acinetobacter* antibiotic targets

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We sought to identify candidate antibiotic targets as those whose genetic inactivation most rapidly block growth. We developed procedures for generating null mutations in all of the ~500 essential *A. baylyi* genes using natural transformation, and then analyzed how rapidly the mutations blocked growth. The results made it possible to rank the essential genes based on how sensitive bacteria were to depletion of their products. The winning functions- those whose inactivation blocked growth the fastest- were involved in ATP generation (proton ATPase), DNA replication (DnaA and ribonucleotide reductase) and protein synthesis (ribosomal proteins).



O8-3: Genetics of *Acinetobacter baumannii* surface polysaccharides reveals insights into cell surface diversity useful for epidemiology and therapeutics research

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Capsular polysaccharide (CPS) and lipooligosaccharide (LOS) are a critical virulence determinants for the globally important species, *Acinetobacter baumannii*. Genes that direct the synthesis and assembly of the CPS are generally clustered at the K locus (KL), whereas genes for the outer-core (OC) oligosaccharide, the most variable portion of the LOS, are located at the OC locus (OCL) [1]. Our team has previously demonstrated the use of K and OC loci diversity for tracing local and global spread of *A. baumannii* clonal lineages. However, as we transition into a genomics era where whole-genome sequencing is an accessible standard for research, the availability of open access computational databases to extract interpretable, actionable information about these genomic regions is critical. Here, we present a complete compendium of >135 KL and >15 OCL full annotated gene clusters available as curated reference databases, which are compatible with the computational tool, 'Kaptive' [2], accessible via a web platform (<http://kaptive.holtlab.net/>) and also source code (<https://github.com/katholt/Kaptive>). These databases integrate details relating to a large body of work directed by our team, providing additional information on the role of specific genes and the carbohydrates that may be synthesised for each structure, identified through the correlation of genetics with the polysaccharide structures produced by more than 50 *A. baumannii* isolates. This work has identified many unique features of *A. baumannii* surface polysaccharides and interesting evolutionary mechanisms directing polysaccharide variation, which are critical implications for epidemiological studies, as well as vaccine and phage research. We tested the databases in an analysis of 3417 genome sequences available on NCBI, which confidently assigned known KLs to 2485 genome sequences, and OCLs to 2302 genome sequences, indicating that the remaining sequences had a novel KL or OCL, or were assemblies of poor quality. This analysis revealed more extensive cell surface diversity than previously expected, expanding the repertoire of tools available for precise epidemiological tracking, further enhancing our understanding of how *A. baumannii* circulates both locally and globally.

[1] J.J. Kenyon, R.M. Hall. Variation in the complex carbohydrate biosynthesis loci of *Acinetobacter baumannii* genomes. PLoS One. 8 (2013), e62160.

[2] R.R. Wick, E. Heinz, K.E. Holt, K.L. Wyres. Kaptive Web: User-friendly capsule and lipopolysaccharide serotype prediction for Klebsiella genomes. J. Clin. Microbiol. 56 (2018), e00197-18.

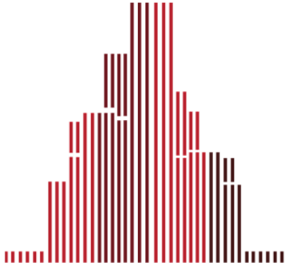
O8-4: Artilysin® - the ingenious way to control bacteria

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Antibiotic resistance has latterly been a growing threat in human health. Nowadays, infections with antibiotic resistant bacteria are one of the most frequent causes of death and also a financial burden for the world's healthcare system. With Artilysin®s, a new class of antibacterial proteins with a new mode-of-action are present. Artilysin®s are optimized, engineered fusions of selected endolysins with specific outer membrane-destabilizing moieties that are able to pass the outer membrane of Gram-negative pathogens and destabilize the peptidoglycan of the cell wall. Subsequently, cells burst due to the high osmotic pressure. Because of their novel mode of action, Artilysin®s are capable to kill both, persistent and multi-drug resistant bacterial cells. Moreover, the molecules come along with a clearly reduced risk of resistance development which was not observed so far. Additionally, no occurrence of any cross-resistance to traditional antibiotics is known.

Acinetobacter baumannii is a Gram-negative, opportunistic pathogen which has become one of the most important pathogens responsible for hospital-acquired infections. Formerly, due to the urgent need of effective drugs, colistin found its way into the markets as the only therapeutic option in many cases. With the treatment of carbapenem-resistant *Acinetobacter baumannii* infections, colistin resistance is emerging resulting in pan-drug resistant isolates. In compassionate use cases, Artilysin®s were shown to be effective against various Gram-negative species on infectious wounds without any side effects promoting wound-healing, reduction of pain and raises quality of living again.



Poster Presentations

P1-1: Evolution of a Clade of *Acinetobacter baumannii* Global Clone 1, Lineage 1 via Acquisition of the *oxa23* Carbapenem Resistance Gene and Dispersion of ISAb_{a1}

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Acinetobacter baumannii causes a range of hospital-acquired infections and antibiotic resistance is a critical problem, particularly when resistance genes are acquired by members of successful globally-distributed clones e.g. global clone 1 (GC1) [1-3]. Here, we investigated the evolution of an expanding sub-clade of multiply antibiotic resistant GC1 associated with carbapenem and aminoglycoside resistance.

Twenty-seven strains belonging to a specific clade of GC1 were identified, 3 in our collection and 24 in GenBank, using a range of criteria including the carriage of the Tn6168 transposon [4], carrying the ISAb_{a1}-*ampC* structure, responsible for resistance to 3rd generation cephalosporins, in a specific chromosomal location, and a specific Outer Core oligoaccharide, OCL3. The genome sequence of the representative of Australian isolates, which was also resistant to carbapenems, was determined using Illumina HiSeq and PacBio long-read technology. A range of bioinformatics tools was used to examine the context of resistance genes, distribution of the chromosomal ISAb_{a1} copies, and phylogeny.

Bayesian analysis showed that the Tn6168/OCL3 clade arose in the late 1990s, from an ancestor that had already acquired resistance to third generation cephalosporins and fluoroquinolones. Between 2000 and 2002, this clade further diverged into distinct sub-clades by insertion of AbaR4 (carrying the *oxa23* carbapenem resistance gene) at a specific chromosomal location in one group, and a phage genome in the other. Both subgroups show evidence of ongoing evolution of resistance loci and ISAb_{a1} dispersal. Most concerning, this includes introduction of the *armA* aminoglycoside resistance gene via AbGR13, acquired from a GC2 isolate.

Our analysis revealed the complexity of genetic events leading to resistance to multiple antibiotics in the Tn6168/OCL3 clade of GC1. Comparison of IS insertions sites with the dated phylogeny shows ISAb_{a1} first entered this clade in around mid 90s with the cephalosporin resistance transposon Tn6168 and has since expanded in both subclades. It also revealed multiple routes for the acquisition of the *oxa23* carbapenem resistance gene.

[1] M. Hamidian, Hall R. M. The AbaR antibiotic resistance islands found in *Acinetobacter baumannii* global clone 1 - Structure, origin and evolution. *Drug Resist Updat.* 2018;41:26-39.

[2] K. E. Holt, Kenyon J. J., Hamidian M., Schultz M. B., Pickard D. J., Dougan G., Hall R. M. Five decades of genome evolution in the globally distributed, extensively antibiotic-resistant *Acinetobacter baumannii* global clone 1. *Microb Genom.* 2016 23;2:e000052.

[3] M. D. Adams, Chan E. R., Molyneaux N. D., Bonomo R. A. Genome wide analysis of divergence of antibiotic resistance determinants in closely related isolates of *Acinetobacter baumannii*. *Antimicrob Agents Chemother.* 2010;54:3569-77.

[4] M. Hamidian, Hall R. M. Tn6168, a transposon carrying an ISAb_{a1}-activated *ampC* gene and conferring cephalosporin resistance in *Acinetobacter baumannii*. *J Antimicrob Chemother.* 2014;69:77-80.

P1-3: Comparative analysis of the two *Acinetobacter baumannii* multilocus sequence typing (MLST) schemes

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Acinetobacter species assigned to the *Acinetobacter calcoaceticus-baumannii* (Acb) complex are Gram-negative bacteria responsible for a large number of human infections. The population structure of Acb has been studied using two 7-gene MLST schemes, introduced by Bartul and coworkers (Oxford scheme) and by Diancourt and coworkers (Pasteur scheme). The schemes have three genes in common but underlie two coexisting nomenclatures of sequence types and clonal complexes, which complicates communication on *A. baumannii* genotypes. The aim of this study was to compare the characteristics of the two schemes to make a recommendation about their usage. Using genome sequences of 730 strains of the Acb complex, we evaluated the phylogenetic congruence of MLST schemes, the correspondence between sequence types, their discriminative power and genotyping reliability from genomic sequences. In silico ST re-assignments highlighted the presence of a second copy of the Oxford *gdhB* locus, present in 553/730 genomes that has led to the creation of artefactual profiles and STs. The reliability of the two MLST schemes was tested statistically comparing MLST-based phylogenies to two reference phylogenies (core-genome genes and genome-wide SNPs) using topology-based and likelihood-based tests. Additionally, each MLST gene fragment was evaluated by correlating the pairwise nucleotide distances between each pair of genomes calculated on the core-genome and on each single gene fragment. The Pasteur scheme appears to be less discriminant among closely related isolates, but less affected by homologous recombination and more appropriate for precise strain classification in clonal groups, which within this scheme are more often correctly monophyletic. Statistical tests evaluate the tree deriving from the Oxford scheme as more similar to the reference genome trees. Our results, together with previous work, indicate that the Oxford scheme has important issues: *gdhB* paralogy, recombination, primers sequences, position of the genes on the genome. While there is no complete agreement in all analyses, when considered as a whole the above results indicate that the Pasteur scheme is more appropriate for population biology and epidemiological studies of *A. baumannii* and related species and we propose that it should be the scheme of choice during the transition towards, and in parallel with, core genome MLST.

P1-4: Delineation of a novel environmental phylogroup of the genus *Acinetobacter* encompassing *Acinetobacter terrae* sp. nov., *Acinetobacter terrestris* sp. nov. and three tentative species

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In May 2019, the genus *Acinetobacter* comprised 60 validly published species names (including four pairs of heterotypic synonyms) and two effectively published species names awaiting validation (<https://apps.szu.cz/anemec/Classification.pdf>). Even though this classification sufficiently covers *Acinetobacter* spp. isolated from humans, the knowledge of the taxonomic diversity of acinetobacters confined to non-human environments is limited.

This study aimed to define the taxonomic position and structure of a novel, phenetically unique group of 26 *Acinetobacter* strains, provisionally designated Taxon 24 (T24). The strains were recovered from soil and freshwater ecosystems (n=21) or animals (n=5) in Czechia, England, Germany, the Netherlands and Turkey between 1993 and 2015. The strains were non-glucose-acidifying, nonhemolytic, nonproteolytic, growing at 32°C and on acetate and ethanol as single carbon sources, but not on 4-hydroxybenzoate and mostly not at 37°C. Their whole-genome sequences were 3.0–3.7 Mb in size, with GC contents of 39.8–41.3%. Based on core genome phylogenetic analysis, the 26 strains formed a distinct clade within the genus *Acinetobacter*, with strongly supported subclades termed T24A (n=11), T24B (n=8), T24C (n=2), T24D (n=3) and T24E (n=2). Internal genomic ANIb values for these subclades were >94.7%, while the ANIb values between them were <92.3%. The results of MALDI-TOF MS-based analyses were in good but not complete agreement with this classification. The five subclades differed from each other in the results of 1–6 carbon source assimilation tests.

Given the genomic and phenotypic distinctness and internal coherence, quantity and geographically diverse origin of T24A and T24B, we propose the respective names *Acinetobacter terrae* sp. nov. and *Acinetobacter terrestris* sp. nov. for these taxa. We conclude that these two species together with the other T24 strains represent a novel, widely dispersed *Acinetobacter* clade primarily associated with terrestrial ecosystems.

P1-5: Evading host defences: Genome sequence analysis of the environmental *Acinetobacter baumannii* NCIMB8209 strain indicates extensive insertion sequence-mediated genome remodelling with loss of exposed cell structures and defensive mechanisms

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Acinetobacter baumannii currently represents an important opportunistic pathogen of obscure reservoirs outside the clinical setting. Here we traced the origins of the collection strain *Acinetobacter sp.* NCIMB8209 to an isolate first reported in 1944 in the U.S.A. NCIMB8209 was isolated from the enriched microbiota responsible for the aerobic decomposition of guayule, a resinous desert shrub. Whole-genome sequencing analysis indicated the presence of a 3.9 Mb chromosome and a plasmid of 134 kb. Phylogenetic analysis based on core genes and a chromosomal *bla*_{OXA-51}-type gene confirmed NCIMB8209 affiliation to *A. baumannii*. The NCIMB8209 chromosome contains 7 genomic islands (GI) and 5 regions encompassing phage-related genes. Remarkably, 93 insertion sequences (IS) were found in its genome, 15 of them correspond to novel mobile elements. No antimicrobial resistance islands were identified, agreeing with a general antimicrobial susceptibility phenotype including to folate synthesis inhibitors. In contrast to its companion, the environmental strain NCIMB8208/DSM30011 [1], NCIMB8209 displayed low virulence in both the *Galleria mellonella* and *Caenorhabditis elegans* infection models. Moreover, this strain lacks GIs providing defences against biological aggressors such as Type 6 secretion systems and corresponding toxin genes. NCIMB8209 harbours many genes linked to persistence and virulence in pathogenic *A. baumannii* strains, but many of them encoding external structures are interrupted by IS. Searching for catabolic genes and metabolic assays revealed several clusters involved in the degradation of plant defence substances, pointing to alternative environmental niche(s) for this species. These results suggest that the disruption in NCIMB8209 of exposed structures likely recognized by host defences most probably resulted from the adaptation of this particular *A. baumannii* strain to a specific environmental niche. Moreover, they also indicated that the reported genetic plasticity of *A. baumannii* represents an intrinsic characteristic of this species, having evolved prior to its adaptation to the clinical environment.

[1] Repizo GD, Viale AM, Borges V, Cameranesi MM, Taib N, Espariz M, Brochier-Armanet C, Gomes JP, Salcedo SP. The environmental *Acinetobacter baumannii* isolate DSM30011 reveals clues into the pre-antibiotic era genome diversity, virulence potential, and niche range of a predominant nosocomial pathogen. *Genome Biol Evol.* **9** (2017), 2292-2307

P1-6: The effects of natural competence on the spatiotemporal evolution of *Acinetobacter* on antibiotic landscapes

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Acinetobacter baylyi is capable of twitching motility and natural transformation using a Type IV pilus [1]. Natural transformation allows for foreign DNA acquisition, which helps drive evolution within a population [2].

We are using a spatiotemporal analysis in antibiotic landscapes to visualise the effects of natural competence in strain ADP1. We are observing evolution of tetracycline resistance using an adapted microbial evolution growth arena (MEGA)-plate in which very large dishes with substantial antibiotic gradients are inoculated with ADP1 at one end, allowing them to twitch and selecting for increasingly resistant mutants. To see an animation of our plate design, please see <https://tinyurl.com/Megaplate2>. We are comparing the emergence of resistant mutants in wild type populations with that in *comA* populations because while cells lacking *comA* have a mild twitching defect, they are not at all transformable. ComA forms the inner membrane channel that allows DNA to enter the cytoplasm. We hypothesize that the evolution of *comA* mutants will likely be similar to that observed for non-transformable *E. coli*, whereas the evolution of wild type cells will be different because of the movement of genes that confer resistance.

[1] Leong CG, Bloomfield RA, Boyd CA, Dornbusch AJ, Lieber L, Liu F, Owen A, Slay E, Lang KM, Lostroh CP. 2017. The role of core and accessory type IV pilus genes in natural transformation and twitching motility in the bacterium *Acinetobacter baylyi*. *Plos One* **12**.

[2] Hülter N, Sørum V, Borch-Pedersen K, Liljegren MM, Utnes ALG, Primicerio R, Harms K, Johnsen PJ. 2017. Costs and benefits of natural transformation in *Acinetobacter baylyi*. *BMC Microbiology* **17**.

P1-7: Genomics of *Acinetobacter baumannii* iron uptake

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Acinetobacter baumannii is a multidrug-resistant nosocomial pathogen. During infection, *A. baumannii* responds to low iron availability imposed by the host through the exploitation of multiple iron-acquisition strategies. To date, six different gene clusters for active iron uptake have been described [1], encoding protein systems involved in: *i*) ferrous iron uptake (Feo); *ii*) heme-uptake (*hemT* and *hemO*), and *iii*) synthesis and transport of the siderophores baumannoferrins (*bfm*), acinetobactin (*bas/bau*) and fimsbactins (*fbs*). Here we collected a compilation of 172 complete *A. baumannii* genomes, also including 52 *de novo* sequenced carbapenem-resistant *A. baumannii* genomes. The dataset, mainly referred to the major International Clones (IC) 1 and 2 (20% IC1, 51% IC2, 29% other), was mined in search for presence and distribution of iron uptake gene clusters. The presence of the *entA* gene, which is located outside of the acinetobactin gene cluster and is essential for the biosynthesis of the acinetobactin precursor 2,3-dihydroxybenzoic acid, was also investigated [2]. Our results indicate that two gene clusters, namely *feo* and *hemT*, are present in all the strains, while *bfm* and *bas/bau* are present in all but the SDF strain. Two *entA* alleles were detected alternatively in 94% and 1% of isolates (ATCC19606-like and ATCC17978-like alleles, respectively), while 2% of isolates carried both alleles, and 3% did not carry or showed mutations in *entA*, as in case of strain AYE. This suggests that ca. 3% of the strains may be incapable of producing acinetobactin. The additional heme uptake system (*hemO*) and the *fbs* gene cluster were detected in 65% and 2% of the strains, respectively. The *hemO* cluster was similarly distributed in IC1 (74%) and IC2 (67%) strains. While all clusters were highly conserved among ICs, major differences were observed at the level of the TonB-dependent receptor of the *hemT* and of the *bas/bau* gene clusters between IC1 and IC2 strains. These findings raise the possibility that differences in receptor specificity of the *hemT* and of the *bas/bau* systems may have influenced the ecological and/or epidemiological success of *A. baumannii* ICs.

[1] L.C.S. Antunes, F. Imperi, K.J. Towner and P. Visca. Genome-assisted identification of putative iron-utilization genes in *Acinetobacter baumannii* and their distribution among a genotypically diverse collection of clinical isolates. *Res Microbiol* **162** (2011), 279-284.

[2] W.F. Penwell, B.A. Arivett and L.A. Actis, The *Acinetobacter baumannii entA* gene located outside the acinetobactin cluster is critical for siderophore production, iron acquisition and virulence. *PLoS ONE* **7**(2012), e36493.

P1-8: Evaluation of clonality by Multiple-Locus Variable number tandem repeat Analysis (MLVA) of *Acinetobacter baumannii* strains of human and animal origin

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Acinetobacter baumannii strains are frequently involved in hospital outbreaks affecting more often immunocompromised patients. Many outbreaks occur in a clonal manner: most of them are linked to the Europeans clones I-III and, since the strains involved are MDR (Multi- Drug Resistant), the available therapies are often limited. Nosocomial infections in veterinary medicine is an emerging concern, but the role of *Acinetobacters* in hospitalized animals is poorly studied.

The aim of this study is to compare by MLVA, *A. baumannii* strains isolated from companions animals with strains isolated from hospital patients or resident in long term care facilities (LTCF).

Twenty *A. baumannii* animal strains belonging to “Istituto Zooprofilattico delle Venezie” collection, 15 human strains isolated in Verona hospital and 4 from LTCF in Verona area, were studied.

Antimicrobial susceptibility to aminoglycosides, cephalosporins, carbapenems and colistin were tested by microdilution method and interpreted according to the EUCAST breakpoints.

All isolates were characterized by the PCR to search for genes that code for the major carbapenemases and Aminoglycoside Modifying Enzymes (AMEs) produced by *A. baumannii*.

MLVA was used to determine the clonal relatedness between strains. Human strains were analyzed also by MLST to determine the international lineage clone.

According to the MICs results all human strains were classified as multidrug resistant, showing resistance to all antibiotics tested except to colistin. Strains isolated from animals resulted sensitive to almost all antibiotics.

Carbapenemase OXA-23 and AMEs were detected both in the majority of human isolates.

MLVA profile analysis results have demonstrated a low correlation between animal and human strains. *A. baumannii* strains isolated from animal showed heterogeneity also if compared between them, indeed human strains isolated in health care structure showed clonal relationships and MLST evidence that they belong to the international clone II. Our human isolates confirmed the presence in the ICII of *bla*_{OXA23} and *armA*.

This preliminary study showed no evidence of genetic relatedness between *A. baumannii* strains of animal and human origin and also a different susceptibility profile to antibiotics.

P1-9: Characterization of *Acinetobacter* spp. isolated from input (livestock manure) and output samples of German biogas plants

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The clinical relevance of multidrug resistant (MDR) *Acinetobacter* has significantly increased [1]. Livestock is one source of MDR bacteria because they are highly exposed to several antibiotics [2]. The application of manure as input material to biogas plant is often discussed as biotechnological barrier to prevent the release of manure-associated resistant bacteria [3, 4]. This may also alter the release of *Acinetobacter* spp. To the best of our knowledge, the transmission of *Acinetobacter* species from livestock via manure into the environment was not studied in detail yet. Here we studied the presence of *Acinetobacter* species by an *Acinetobacter* specific 16S rRNA gene sequence targeting qPCR approach in input and output material of 15 German biogas plants. The concentration was in the range of 10^6 to 10^8 in input and 10^5 to 10^6 16S rRNA gene targets g^{-1} FW (fresh weight) output material, respectively. During a non-targeted cultivation-based study 12 *Acinetobacter* isolates including *A. baumannii* strains were cultured from input and five from output materials, including six strains identified as *A. baumannii*. Here we give a detailed phylogenetic characterization and antibiotic resistance profiling of those isolates. Distinct differences were obtained between input and output strains. In addition, the epidemiological relevance of *A. baumannii* was studied by a comparative genome and MLST based approach. In addition the genome of a novel species, which was isolated from an output sample in the presence of a carbapenem is described in detail. This study gives a first detailed characterization of *Acinetobacter* spp. released via manure and biogas plant output material into the environment.

[1] C. Willyard. Drug-resistant bacteria ranked. *Nature* 2017;543:15.

[2] Chee-Sanford JC, Mackie RI, Koike S et al. Fate and Transport of antibiotic residues and antibiotic resistance genes following land application of manure waste. *J Environ Qual* 2009;38:1086.

[3] Manyi-Loh CE, Mamphweli SN, Meyer EL et al. Microbial anaerobic digestion (bio-digesters) as an approach to the decontamination of animal wastes in pollution control and the generation of renewable energy. *Int J Environ Res Public Health* 2013;10:4390–417.

[4] Saunders O, Harrison J, Fortuna AM et al. Effect of anaerobic digestion and application method on the presence and survivability of *E. coli* and fecal coliforms in dairy waste applied to soil. *Water, Air, Soil Pollut* 2012;223:1055–63.

P1-10: Tracing protein interaction networks across species and through time

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Reconstructing the evolutionary trajectory that turned an ancestral environmental bacterium into the human pathogen *Acinetobacter baumannii*, is essential for an informed treatment of induced infections. Comparative genomics approaches to assess the evolutionary relationships and functional similarities of proteins encoded in contemporary *A. baumannii* genomes across large and phylogenetically diverse taxon collections form the basis of such analyses. With the number of sequenced *A. baumannii* strains ever increasing, the field is in need of scalable, robust, and intuitive methods to fully explore bacterial functional diversity.

Here, we present a software suite to rapidly trace proteins and their functions across species and through time. HaMStR-OneSeq[1] facilitates the dynamic generation of feature-aware phylogenetic profiles for a set of seed proteins across user-defined taxon collections of any size. The software integrates a targeted ortholog search with an assessment of the pair-wise feature architecture similarity (FAS) between the seed and its orthologs. For the ortholog search HaMStR-OneSeq applies a profile hidden Markov model based approach, which scales linearly with the number of search taxa. For the FAS scoring, the software assesses identity, copy number- and positional similarity of shared features, such as functional protein domains, between the seed protein and each of its orthologs. The resulting feature-aware phylogenetic profiles can be visualized, interactively explored, and analyzed in PhyloProfile[2]. PhyloProfile allows to dynamically adapt the resolution from an overview analysis across hundreds of proteins and hundreds of taxa to the pair-wise comparison of individual proteins at the level of their domain architectures without the need to modify the input data. Next to the visualization of phylogenetic profiles, PhyloProfile provides a rich set of analysis functions. These include, among others, (i) the identification of proteins contributing to the same metabolic pathway, (ii) the estimation of gene ages, and (iv) the search for proteins with a lineage specific change in their domain architectures, which is indicative for a change in protein function. The HaMStR-suite is a novel and powerful software solution to trace and assess functional innovation in the genomes of pathogenic bacteria.

[1] <https://github.com/BIONF/HaMStR>

[2] N.V. Tran, B. Greshake Tzovaras, and I. Ebersberger. PhyloProfile: dynamic visualization and exploration of multi-layered phylogenetic profiles. *Bioinformatics* **34** (2018), 3041-3043.

P1-11: The catalogue of *A. baumannii* virulence factors

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The comprehensive identification and characterization of virulence factors in the genome of a bacterial pathogen is a crucial step in bacterial pathobiology. Current methods rely on comparative approaches, and aim at identifying homologs to a catalogue of known virulence factors. Their predictive power with respect to the virulence characteristics of a particular bacterium ultimately depends on the comprehensiveness and the accuracy of the underlying virulence database.

Here, we present an up-to-date collection of *A. baumannii* virulence factors. Our database currently provides access to a non-redundant list of 476 proteins that are either known or at least suspected to contribute to *A. baumannii* virulence. The database was compiled on the basis of entries in 6 existing virulence database, combined with candidates suggested as virulence factors in the literature. We are currently extending this initial collection of proteins with novel putative virulence factors that emerge from large-scale comparative genomics analysis of *Acinetobacter* genomes. In a first approach, we traced the evolution of the *A. baumannii* ATCC19606 gene set across more than 3,000 representatives of the genus *Acinetobacter*. Specifically, we screened for proteins that either emerged on the evolutionary lineage leading to *A. baumannii*, or which changed their domain architecture on this lineage, which indicates a lineage-specific change in function. Interestingly, we found among the novel virulence factor candidates, three proteins involved in host cell adhesion: Ata, the type IV pilus tip ComC, and the fimbrial tip CsuE. These proteins are known to play a role in biofilm formation, pathogen-host interactions, and/or motility[1,2,3].

In a complementary approach, we are developing a software pipeline to ease a standardized search for virulence factor in hitherto uncharacterized bacterial genomes. Profile hidden Markov Models of the individual virulence factors paired with a targeted and feature-aware ortholog search[4] will provide, for a user-defined input genome, a rapid assessment of its virulence characteristics.

[1] Averhoff B, Friedrich A. 2003. Type IV pili-related natural transformation systems: DNA transport in mesophilic and thermophilic bacteria. *Arch Microbiol.* 180(6):385-393.

[2] Pakharukova N, Garnett JA, Tuittila M, Paavilainen S, Diallo M, Xu Y, Matthews SJ, Zavialov AV. 2015. Structural insight into archaic and alternative chaperone-usher pathways reveals a novel mechanism of pilus biogenesis. *PLoS Pathog.* 11(11):22.

[3] Weidensdorfer M, Ishikawa M, Hori K, Linke D, Djahanschiri B, Iruegas R, Ebersberger I, Riedel-Christ S, Enders G, Leukert L, Kraiczky P, Rothweiler F, Cinatl K, Berger J, Hipp K, Kempf VAJ, Göttig S. 2019. The *Acinetobacter* trimeric autotransporter adhesin Ata controls key virulence traits of *Acinetobacter baumannii*. *Virulence.* 10(1):68-81.

[4] <https://github.com/BIONF/HaMStR>



P1-12: The use of genomic-scale models in describing the *A. baumannii* infection process

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Genomic-scale models capture the relationship between genotype and an organism's phenotype. They model the constraints that are imposed on the phenotype of a biochemical system jointly by physiochemical laws, genetics and the environment[1]. GEMs have found numerous applications throughout the recent years ranging from network characterization[2], metabolic-engineering[3], evolution[4], medical drug targeting[5] to studying the interactions of microbial communities. Multiple methods were developed that integrate large-scale transcriptomic data into the model. Effectively, this creates a condition specific model that is able to predict biological phenotypes more accurately. Ultimately, GEMs help to identify bottlenecks in the metabolism of a pathogenic bacterium that point out novel drug-target candidates.

Here, we will present our approach to adapt the publicly available GEM of *Acinetobacter baumannii* ATCC19606[7] to describe the metabolic properties of this strain during planktonic growth, and during infection of human cell lines or of larvae of *Galleria mellonella*. The integration of transcriptomic data from different experimental time points will be used to step-wise monitor the *A. baumannii* infection program on the level of the metabolome. On the basis of this data, we will use the GEM to identify genes that are essential for the infection process. Moreover, we will focus on the comparison of the metabolic changes that are associated with either infecting human cells or the insect larvae. This will shed light on the question to what extent conclusions drawn from the analysis of an insect infection model generalize to humans.

[1] L. Heirendt, S. Arreckx, T. Pfau, *et al.* (2019) Creation and analysis of biochemical constraint-based models using the COBRA Toolbox v.3.0. *Nat Protoc*, **14** (2019), 639-702.

[2] M. A. Oberhardt, B. Ø. Palsson, and J. A. Papin (2009) Applications of genome-scale metabolic reconstructions. *Mol Syst Biol*. **5** (2009)

[3] A. R. Zomorrdi, P. F. Suthers, S. Ranganathan, and C. D. Maranas (2012) Mathematical optimization applications in metabolic networks. *Metab Eng*. **14** (2012), 672-686

[4] B. Papp, R. A. Notebaart, and C. Pál (2011) Systems-biology approaches for predicting genomic evolution. *Nat Rev Genet*. **12** (2011), 591-602

[5] A. K. Chevali, K. M. D'Auria, E. L. Hewlett, R. D. Pearson, and J. A. Papin (2012) A metabolic network approach for the identification and prioritization of antimicrobial drug targets. *Trends Microbiol*. **20** (2012) 113-123

[6] K. Zengler, and B. Ø. Palsson (2012) A road map for the development of community systems (CoSy) biology. *Nat Rev Microbiol*. **10** (2012), 366-372

[7] Y. Zhu, J. Zhao, M. H. M. Maifiah, T. Velkov, F. Schreiber, and J. Li (2019) Metabolic Responses to Polymyxin Treatment in *Acinetobacter baumannii* ATCC19606: Integrating Transcriptomics and Metabolomics with Genome-Scale Metabolic Modeling. *mSystems*. **4** (2019)

P1-14: Diversity of *Acinetobacter baumannii* strains and bacteriophage-mediated spread of the OXA-23 carbapenemase in Alexandria, Egypt

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Carbapenem-resistant *A. baumannii* are prevalent in low and middle income countries such as Egypt, but little is known about the molecular epidemiology and mechanisms of resistance in these settings. Here we characterise carbapenem-resistant *A. baumannii* from Alexandria, Egypt, and place it in a regional context.

57 carbapenem-resistant isolates from Alexandria Main University Hospital, Egypt, collected between 2010 and 2015 were genome sequenced using Illumina technology. Genomes were *de novo* assembled, annotated, and SNPs called. Genomes for 36 strains from the Middle East region were downloaded from GenBank. Core gene complement was determined using Roary, and analyses of recombination were performed in Gubbins. MLST sequence type and antibiotic resistance genes were identified.

The majority of Egyptian strains belonged to one of 3 major clades, corresponding to Pasteur MLST clonal complex (CC) 1 (14 strains), CC2 (25 strains) and ST158 (10 strains). Strains belonging to ST158 have only been reported three times, once each in Turkey, Iraq, and Egypt, and may represent a region-specific lineage. Forty-nine strains carried a *bla*_{OXA-23} gene, 6 carried *bla*_{NDM-1}, and one carried *bla*_{NDM-2}. The *bla*_{OXA-23} gene was located on a variety of different mobile elements, with Tn2006 predominant in CC2 strains, and Tn2008 predominant in other lineages. Of particular concern, in 9 of the 11 CC1 strains carrying *bla*_{OXA-23}, the carbapenemase gene was located in a bacteriophage phiOXA, previously identified only once before in a CC1 clone from the US military. Within CC2 and CC1, strains were hugely diverse, with a median SNP distance of 4,596 and 3,160 SNPs respectively, suggesting *A. baumannii* is endemic within the healthcare environment or population. ST158 strains were closely related, with a median distance of 9 SNPs. There was substantial evidence for recombination within clonal complexes that was not randomly distributed, with hotspots in regions such as the K locus (capsule) and the OC locus (LPS component). Patterns of recombination were similar between CC2 and CC1 strains.

The carbapenem-resistant *A. baumannii* population in Alexandria Main University hospital is very diverse, and indicates an endemic circulating population, including a region-specific lineage. The major mechanism for *bla*_{OXA-23} dissemination in CC1 strains appears to be a bacteriophage, presenting new concerns about the ability of these carbapenemases to spread throughout the bacterial population.

P2-1: Fatal attraction: *Acinetobacter baumannii* exploits carcinoembryonic antigen-related cell adhesion molecules (CEACAMs) for cellular adherence

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Bacterial cell adherence is an essential first step in establishing colonization and, eventually, infection. *Acinetobacter baumannii* is a life-threatening bacterium mainly responsible for ventilator-associated pneumonia. It adheres and invades host cells by the interaction between the bacterial phosphorilcholine-containing outer membrane protein and the host platelet-activating factor receptor [1]. Receptor engagement triggers a cascade of pathways that leads to bacterial internalization [1]. Interestingly, several respiratory human pathogens engage carcinoembryonic antigen-related cell adhesion molecules (CEACAMs) for host adhesion and invasion. CEACAMs are immunoglobulin-related glycoproteins that trigger cell–cell communication, inflammation and cancer progression. CEACAM1, CEACAM5, and CEACAM6 are found on various epithelial cell types, while others such as CEACAM8 are expressed sole on granulocytes. In this study, we have hypothesized that *A. baumannii* could exploit CEACAMs to increase adhesion to epithelia. By pull-down and fluorescence immunostaining approaches with specific antibodies, we demonstrated that *A. baumannii* strain AB5075 associated with purified CEACAM1, CEACAM5 and CEACAM6 receptor proteins, but not with CEACAM8. Deletion of the N-terminal IgV-like domain of recombinant CEACAM1 protein abrogated the interaction, highlighting the importance of this domain for interaction. The adhesion rates of strain AB5075 were significantly increased using stably transfected lung epithelial A549 cells individually expressing CEACAM1, CEACAM5 and CEACAM6 in comparison to A549 transfected with the empty vector or CEACAM8. Pull-down experiments performed with proteolytically digested *A. baumannii* cells abolished CEACAM specific interaction, indicating the involvement of bacterial proteinaceous element(s). Incubation of CEACAM transfected A549 cells with an inhibitor of N-glycosylation (i.e. tunicamycin) showed no decrease in the adhesion rates of strain AB5075, suggesting that bacterial-CEACAM-interaction does not relies on CEACAM major surface-associated saccharides. Since increased epithelial cell adhesion creates the potential for invasion, experiments are in progress to assess the steps following the binding of *A. baumannii* to CEACAM on the cell surface.

[1] Smani Y, Docobo-Pérez F, López-Rojas R, Domínguez-Herrera J, Ibáñez-Martínez J, Pachón J. Platelet-activating factor receptor initiates contact of *Acinetobacter baumannii* expressing phosphorylcholine with host cells. *J Biol Chem.* **287** (2012):26901-26910.

P2-2: Outer membrane protein biogenesis in *Acinetobacter baumannii*: links to virulence and drug resistance

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The number and severity of infections with multidrug-resistant Gram negative pathogens has been increasing during the last decades with highest incidence at intensive care units (ICUs). One of the pathogens ranked with highest priority for development of new therapies are carbapenem-resistant strains of *Acinetobacter baumannii* (*Ab*) [1]. For *Yersinia enterocolitica* (*Ye*) and *Pseudomonas aeruginosa* (*Pa*), our group has previously validated a number of genes (*surA*, *degP*, *bamB*, *bamC*, *skp*) involved in the biogenesis of outer membrane proteins for their potential as novel targets to develop therapeutics that reduce virulence and possibly break resistance. As we had discovered significant differences of the importance of some of our candidate genes in *Ye* and *Pa*, we wanted to determine their relevance also using *Ab*. To this end, we analysed K.O. mutants for *surA*, *degP*, *bamB*, *bamC* and *skp* of a highly virulent multidrug-resistant *Ab* strain (*AB5075*). A *surA* knockout in *Ye* or a conditional *surA* knockout in *Pa* resulted in reduced serum resistance, enhanced susceptibility to antibiotics and overall reduced virulence of both pathogens [2,3]. Surprisingly, the *surA* knockout mutant of *Ab* only had very weak phenotypes, but a strain lacking *bamC* showed interesting phenotypes. Our work thus demonstrates the necessity of exhaustive target validation in multiple species.

[1] Dijkshoorn, L., et al. (2007). "An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*." *Nat Rev Microbiol* **5**(12): 939-951.

[2] Weirich, J., C. Bräutigam, M. Mühlenkamp, M. Franz-Wachtel, B. Macek, I. Meuskens, M. Skurnik, K. Leskinen, E. Bohn, I. Autenrieth and M. Schütz (2017). "Identifying components required for OMP biogenesis as novel targets for anti-infective drugs." *Virulence* **8**(7): 1170-1188.

[3] Klein, K., M. S. Sonnabend, L. Frank, K. Leibiger, M. Franz-Wachtel, B. Macek, T. Trunk, J. C. Leo, I. B. Autenrieth, M. Schütz and E. Bohn (2019). "Deprivation of the Periplasmic Chaperone SurA Reduces Virulence and Restores Antibiotic Susceptibility of Multidrug-Resistant *Pseudomonas aeruginosa*." *Front Microbiol* **10**: 100.

P2-3: Comparison between intranasal inoculation, intratracheal instillation and oropharyngeal aspiration techniques in inducing pneumonia in mice with *A. baumannii* MDR strains

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Infectious pneumonia induced by multidrug resistant *A. baumannii* (*A.b.* MDR) strains is among the most common and deadly forms of healthcare acquired infections [1]. Over the years, several animal models of *A.b.* pneumonia have been developed to test efficacy of novel therapeutics. However, since only a self-limiting pneumonia with no or limited local bacterial replication was frequently obtained, different strategies have been put in place to increase host susceptibility to *A.b.* and bacterial virulence. The technique employed to challenge the animals is one of the most important factors to be considered. Intranasal inoculation is an easy very popular technique that can be used repeatedly. However, a large number of bacteria could be retained in upper respiratory tract or swallowed into the gastrointestinal tract [2]. Intratracheal instillation delivers pathogens directly into the lungs of mice with high reproducibility and accuracy but requires high technical expertise and expensive equipment. The oropharyngeal aspiration technique is an effective, inexpensive and reliable inhalation method based on the natural aspiration reflex of the mouse [3].

In this study, the timecourse of pneumonia induced by 10⁶ CFU/mouse of two *A.b.* MDR clinical strains isolated from sputum administered using three different techniques was recorded during 26 hours of infection.

Results show that the two strains were not able to colonize the lungs when inoculated via intranasal route since no increase of bacterial load was recorded with respect to the start of therapy. By contrast, the bacterial counts of intratracheally infected mice increased significantly by 1.5-2 Log₁₀ CFU/lungs during the 26 hours of monitoring, thus highlighting the ability of these strains to establish pneumonia when the bacterial suspension was directly instilled in the lower airways. Finally, oropharyngeally challenged animals showed an increase of lung bacterial burden comparable to that found in the intratracheally treated mice and, additionally, a substantial decrease of the intra-group variability was recorded. Findings were supported by histopathological examination of lungs and biomarkers of inflammation.

To conclude, both intratracheal instillation and oropharyngeal aspiration techniques are suitable methods to induce pneumonia in mice with difficult MDR *A.b.* strains. In particular, oropharyngeal aspiration is an easy and consistent method suitable for initiating robust infections with low variance.

[1] L. L. Maragakis and T. M. Perl. *Acinetobacter baumannii*: Epidemiology, Antimicrobial Resistance, and Treatment Options. Clin Infect Dis **46** (2008), 1254–1263.

[2] M. A. Miller, J. M. Stabenow, J. Parvathareddy, A.J. Wodowski, T.P. Fabrizio, X.R. Bina, L. Zalduondo, J. E. Bina. Visualization of murine intranasal dosing efficiency using luminescent *Francisella tularensis*: effect of instillation volume and form of anesthesia. PLoS One **7** (2012), e31359.

[3] T. B. Nielsen, J. Yan, B. Luna and B. Spellberg. Murine oropharyngeal aspiration model of ventilator-associated and hospital-acquired bacterial pneumonia. JOVE **136** (2018), e57672.

P2-5: Female mice have impaired control of *Acinetobacter baumannii* induced pneumonia that is aggravated in the absence of alveolar macrophages

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Acinetobacter baumannii is an extremely versatile multidrug resistant pathogen with a very high mortality rate. As so, it has become crucial to better understand how the host senses and responds to *A. baumannii* infection. While establishing a non-lethal acute pneumonia model using the highly virulent clinical isolate AB5075, we observed that female mice had significant increases in bacterial burden in the airway, lung and spleen 24 h post-infection compared to infected male mice. Infection using weight matched female and male mice with *A. baumannii* 5075 and ATCC19606 confirmed our initial observation. Analysis of the immune cells in the airway revealed that female mice had reduced numbers of alveolar macrophages (68.4%, $P < 0.01$) and neutrophils (64.5%, $P < 0.01$) in response to *A. baumannii*. Female mice also displayed a significant increase in numerous pro-inflammatory cytokines. It is known that both alveolar macrophages and neutrophils play important roles in controlling *A. baumannii* induced pneumonia. To better understand the role of these immune cells in both sexes depletion experiments were performed using clodronate containing liposomes (alveolar macrophages) and Ly6G neutralizing antibody (neutrophils). Depletion of both cell types resulted in significant increases in bacterial burden in both female and male infected mice. However, the absence of alveolar macrophages led to a significantly higher magnitude of bacterial burden in female compared to male mice (>1500 fold greater, $P < 0.01$). RNA sequencing of whole lung homogenates of clodronate treated female and male mice following a 4 h infection, showed major reductions in the respiratory chain and ribosomal genes, indicating that alveolar macrophages from female mice contribute significantly to energetic requirements and generation of oxidative byproducts. Our data highlights the importance of utilizing both sexes when assessing host immune pathways and points to a crucial role for alveolar macrophages in controlling *A. baumannii* infection in female mice.

P2-6: Imaging of bioluminescent *Acinetobacter baumannii* in a mouse pneumonia model

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Acinetobacter baumannii has emerged as a troublesome pathogen worldwide because of its multidrug-resistance [1]. Novel antibiotics or alternative therapeutic approaches, such as anti-virulence agents and bacteriophages, are needed to overcome antimicrobial resistance of *A. baumannii* [2]. Animal models are essential to analyze bacterial pathogenesis and to develop new drugs in bacterial infectious diseases. Non-invasive bioluminescence imaging has proven useful for *in vivo* real-time monitoring of bacterial infections in animal models [3,4]. However, no bioluminescence imaging assay has been developed to monitor *A. baumannii* infections.

Bioluminescent strains of *Acinetobacter baumannii* ATCC 17978 and its isogenic $\Delta ompA$ mutant were constructed by integrating the *luxCDABE* luciferase gene into the bacterial chromosome [5]. In an acute murine pneumonia model, bioluminescence of the two reporter strains was clearly visible in the lungs 30 min after infection, and the bioluminescent signal increased over 24 h or 48 h. The bioluminescent *A. baumannii* strains show similar pathogenic traits *in vivo* compared to their parent strains [6]. Bioluminescence was correlated with bacterial burden and histopathology in reporter strain-infected mice. We developed bioluminescent reporter strains of *A. baumannii* whose bioluminescence was highly correlated with the bacterial burden and histopathology in mouse lungs. The bioluminescent imaging assay using the *A. baumannii* reporter strains containing the *luxCDABE* gene is a useful tool for the real-time monitoring of *A. baumannii* infection in animal models.

[1] D. Wong, TB. Nielsen, RA. Bonomo, et al. Clinical and pathophysiological overview of *Acinetobacter* infections: a century of challenges. *Clin. Microbiol. Rev.* **30** (2017), 409-447.

[2] SW. Dickey, GYC. Cheung, M. Otto. Different drugs for bad bugs: antivirulence strategies in the age of antibiotic resistance. *Nat. Rev. Drug Discov.* **16** (2017), 457-471.

[3] MH. Chang, SL. Cirillo, JD. Cirillo. Using luciferase to image bacterial infections in mice. *J. Vis. Exp.* **48** (2011), 2547.

[4] S. Henken, J. Bohling, AD. Ogunniyi, et al. Evaluation of biophotonic imaging to estimate bacterial burden in mice infected with highly virulent compared to less virulent *Streptococcus pneumoniae* serotypes. *Antimicrob. Agents Chemother.* **54** (2010), 3155-3160.

[5] MH. Oh, JC. Lee, J Kim, CH Choi, K Han. Simple method for markerless gene deletion in multidrug-resistant *Acinetobacter baumannii*. *Appl. Environ. Microbiol.* **81** (2015), 3357-3368.

[6] CH. Choi, JS Lee, YC. Lee, et al. *Acinetobacter baumannii* invades epithelial cells and outer membrane protein A mediates interactions with epithelial cells. *BMC Microbiol.* **8** (2008), 216.

P2-7: Microevolution in the major outer membrane protein OmpA of *Acinetobacter baumannii*

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OmpA is the most abundant protein in the outer membrane of *Acinetobacter baumannii*, and has been proposed as a vaccine target. It forms an 8-stranded beta-barrel domain embedded in the outer membrane, four extra-cellular external loops (ELs), and a C-terminal periplasmic domain that binds to cell wall peptidoglycan. *A. baumannii* OmpA proposed functions range from a porin involved in antibiotic resistance, host cell adhesion and invasion, and biofilm formation, representing many different, potentially competing, selective pressures acting upon the protein. Here, we analysed the polymorphisms in *Acinetobacter* OmpA to obtain clues on how the clinical environment may have shaped the evolution of this protein.

Analysis of nearly 250 *Acinetobacter* genomes identified five major groups of *ompA* alleles, named V1 to V5, comprising 50 different alleles coding for 29 different protein variants. Across the protein sequence, 17% of amino acid positions were polymorphic, with polymorphisms concentrated in 5 regions corresponding to the four ELs and the C-terminal end of the protein. Evidence for intra-genic recombination was seen, for example between the V3 and V4 groups at EL1, the V2 and V4 groups at EL4, and a 6 amino-acid indel located at the C-terminal end of the protein. When mapped against a core gene phylogeny, *ompA* alleles were not randomly distributed, with the V1a1 allele almost exclusive to MLST clonal complex 1 (CC1) strains (Pasteur MLST typing scheme), while the V2a1 allele was found in 26/31 CC2 strains. Evidence was found for exchange of *ompA* alleles between different *A. baumannii* lineages, and between different *Acinetobacter* species. Analyses of the substitution patterns of the alleles within V groups found little evidence for positive selection of the ELs, but suggested that non-synonymous substitutions in the transmembrane regions between species may have been selected.

In conclusion, we find a diversity of OmpA alleles in *A. baumannii*, and that major global clones preserved their clone-associated allele, suggesting they confer significant fitness advantages. Lateral gene transfer and recombination appear to drive the evolution of OmpA in this species, such as the acquisition of a C-terminal insertion that may stabilise the bacterial envelope. These data demonstrate that any *ompA* allele that evolves a clinically relevant phenotype can spread within and between *Acinetobacter* species, and that a vaccine based upon OmpA may be limited in its effectiveness.

P2-8: Adhesions of *Acinetobacter baumannii* to immune cells trigger inflammation responses

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Acinetobacter baumannii is currently the most important pathogens associated with nosocomial infections and is characterized by a high prevalence of drug resistance. *A. baumannii* biofilm formation, capsule, and high iron-acquisition capacity are known virulence factors [1-2]. We previously reported that *A. baumannii* escaped from neutrophil extracellular traps (NET) formation and phagocytosis [3]. Furthermore *A. baumannii* adhered to neutrophils and transported together via inducing IL-8 release [4]. However, the interaction between other immune cells and *A. baumannii* is still unclear. Thus, the interactions between mast cells and *A. baumannii* were examined in this study.

Mast cells produced TNF- α and IL-8 when the cells were co-cultured with live *A. baumannii* *in vitro*. TNF- α from *A. baumannii*-infected mast cells showed that neutrophils activation and migration increased in Boyden chamber assays. Gene expression analysis showed that *TNF- α* and *IL-8* mRNA increased in *A. baumannii* -infected mast cells. Scanning electron microscopy showed that *A. baumannii* was tightly adhered to the surface of mast cells. We observed that *A. baumannii* bind to Fc γ RII (CD32) on mast cells. The number of *A. baumannii* adhered on mast cells and TNF- α release decreased by anti CD32 antibody treatment [5]. We also observed LPS derived from *A. baumannii* strongly induced IL-8 production from mast cells. It was suggested that adhesion of *A. baumannii* to immune cells might trigger production of pro-inflammatory cytokines, while avoiding phagocytosis by neutrophils and consequently expanding inflammation.

[1] A. Peleg, H. Siefert, D. Paterson. *Clin.Microbiol.Rev.* **21** (2008), 538-582.

[2] C. Wood, E. Ohneck, R. Edelmann, L. Actis. *Infect. Immun.* **86** (2018)

[3] G. Kamoshida, T. Kikuchi-Ueda, S. Tansho-Nagakawa, R. Nakano, A. Nakano, H. Kikuchi, T. Ubagai, Y. Ono. *J. Infect Chemother* **21** (2015) 43-49.

[4] G. Kamoshida, S. Tansho-Nagakawa, T. Kikuchi-Ueda, R. Nakano, K. Hikosaka, S. Nishida, T. Ubagai, S. Higashi, Y. Ono. *J. Leukocyte. Biol.* **100** (2016)

[5] T. Kikuchi-Ueda, G. Kamoshida, T. Ubagai, R. Nakano, A. Nakano, T. Akuta, K. Hikosaka, S. Tansho-Nagakawa, H. Kikuchi, Y. Ono. *Immunobiology* **222** (2017) 1025-1034

P2-9: Recruitment of complement regulators by biofilm-producing *Acinetobacter baumannii*

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Acinetobacter baumannii belongs to the ESKAPE pathogens known to be the leading bacteria of nosocomial infections worldwide. This particular pathogen is able to produce biofilms on biotic and abiotic surfaces that often results in device-associated infections. Biofilms of *A. baumannii* contribute to antibiotic resistance and help the bacteria to overcome innate immunity. In this study, we investigated the interactions of complement regulators with biofilms in order to elucidate the mechanism by which *A. baumannii* escapes complement-mediated killing.

Several *A. baumannii* strains were screened for their potential to produce biofilms at different temperatures using a crystal violet staining assay. Binding of complement factors such as complement factor H (FH), C4b-binding protein (C4BP), and C1-esterase inhibitor (C1-Inh) to *A. baumannii* biofilms was investigated. Additionally, binding of FH and C4BP was visualized by employing confocal microscopy. Furthermore, we analyzed whether FH and C4BP bound to the surface of formed biofilms retain their complement regulatory activity.

All analyzed *A. baumannii* strains were able to produce biofilm under different conditions. Most strains grown at room temperature showed higher amounts of biofilm production compared to strains grown at 37 °C. Concerning binding of complement regulators FH, C1-Inh, and C4BP two out of eight biofilm-producing strains bound all three regulators and two additional strains solely bound FH. Interestingly, planktonically grown cells did not bind complement regulators at all. Confocal microscopy revealed that complement regulators were preferentially bound at the air-liquid interface, the areas with the highest density of biofilm. Cofactor assays showed that FH and C4BP attached to the biofilm remained functionally active and could degrade complement component C3b and C4b.

The mechanism(s) how biofilm protects *A. baumannii* from innate immunity are not well understood. Here, we demonstrate that biofilm-producing *A. baumannii* strains are able to recruit FH, C4BP and C1-Inh to the surface of the generated biofilm. Functional analyses revealed that FH and C4BP bound to biofilm degrade complement C3b and C4b. Thus, serum resistance of *A. baumannii* is at least in part influenced by their ability to form biofilms. Binding of complement regulators to the surface strongly reduce complement activation and thereby protecting bacterial cells from detrimental effects of complement



P2-10: Role of the Acinetobactin Cluster of *A. baumannii* ATCC 17978 in virulence

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Antibiotic resistance will cause the death of 10 million people in 2050. Searching for targets linked to pathogenicity is needed to develop new therapeutic tools and to stop this pandemic. When an infection takes place, virulence is directly related to the capacity of a microorganism to sequester available iron in the environment. In the case of *A. baumannii* the most-studied siderophore is the acinetobactin.

The present work studies the key role of genes related to biosynthesis, reception and efflux of acinetobactin implicated in iron scavenging from host sources, describing a group of genes directly related to iron-uptake that could work as new therapeutic targets.

The knockout mutants of the *A. baumannii* ATCC 17978 strain lacking all the 18 genes belonging to the acinetobactin cluster, *bas* genes (biosynthesis), *bar* genes (efflux) and *bau* genes (reception), were obtained by double-crossover recombination. Growth rate curves were done in Müeller-Hinton in presence and absence of iron (supplemented with bipyridyl). A motility assay was carried for each mutant in presence/absence of iron. HPLC analyses were performed to detect the siderophore in the supernatant of the mutants. A murine sepsis model was used to evaluate the *in vivo* virulence.

Strains lacking *basG*, *basD*, *basC*, *basB*, *barB* or *barA* genes, showed an acute growth rate decrease. A partial reduction of the growth rate was observed in strains lacking *basA*, *bauB*, *bauE*, *bauC* or *bauD* genes. On the other hand, an important reduction of the motility ability was observed for the strains lacking *basI*, *basJ*, *barA*, *barB*, *basG*, *basD*, *basC*, *basB* and *basA* genes. Also, a partial reduction of this motility was observed for the mutants lacking *basJ*, *basH*, *basF*, *basE*, *bauB*, *bauE* and *bauD* genes. The results of the HPLC analysis showed the complete absence of the siderophore in the supernatant of the cultures of the mutants lacking the latest biosynthesis steps (*basG*, *basC*, *basD* and *basB*) with respect to the other mutants analysed and the parental strain. Mice survival rates were significantly higher in the mutants *basG*, *basD*, *basC* and *basB* mutants (implicated in biosynthesis) and in *bauA*, *barA*, *barB* mutants (implicated in transport), compared to the wild-type strain ATCC 17978.

The genes involved in the latest synthesis steps (*basG*, *basD*, *basC* and *basB*) and in the transport (*bauA*, *barA* and *barB*) of acinetobactin are involved in blood infection, being the first group crucial for the pathogenesis of *A. baumannii* ATCC 17978.

P2-11: Highly pathogenic multidrug-resistant *Acinetobacter baumannii* and experimental treatment with the fourth-generation cephalosporin ceftazidime

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Nosocomial infections caused by multidrug-resistant (MDR) pathogens often have a poor outcome, particularly in intensive care units. *A. baumannii* is a Gram-negative pathogen that causes opportunistic infections; worryingly, MDR *A. baumannii* (MDRA) strains are often resistant to major antibiotics such as amikacin, ciprofloxacin, and imipenem. A fatal MDRA outbreak occurred at Teikyo University Hospital in 2010 [1]. Here, we compared the pathogenicity of *A. baumannii* clinical isolates, including MDRA strains. The greater wax moth *Galleria mellonella* was used to assess the virulence and antibiotic response profiles of carbapenem-resistant *A. baumannii* isolates from this fatal outbreak. Amikacin-resistant *A. baumannii* were more pathogenic in *G. mellonella* than in amikacin-sensitive *A. baumannii*, suggesting that *armA*-positive isolates are generally more virulent against *G. mellonella* larvae. MDRA isolate TK1033 was more virulent than other *A. baumannii* isolates; however, TK1033 was sensitive to the fourth-generation cephalosporin ceftazidime (in addition to minocycline, tigecycline, and polymyxins [colistin and polymyxins B]) both *in vitro* and in the *in vivo* MDRA-*G. mellonella* infection model. Differences in pathogenicity among carbapenem-resistant *A. baumannii* clones are consistent with heterogeneous clinical outcomes. Strain TK1033, isolated frequently during the outbreak, was the most virulent among *A. baumannii* isolates, whereas non-outbreak isolate TK1032 was less virulent. Infection with high-virulence isolates may be more prevalent during outbreaks. These strains may prove valuable for investigating MDRA virulence and for identifying novel therapeutics [2]. To reduce the spread of MDR microorganisms to low-prevalence countries such as Japan, screening of all patients with a history of hospitalization and travel abroad is inevitable [3].

1. Yuji K, Oiso G, Matsumura T, Murashige N, Kami M. Clin. Infect. Dis. **2011**; 52:422–422.
2. Nishida S, Ono Y. Infect. Drug Resist. **2018**; 11:1715–1722.
3. Nishida S, Ishigaki S, Asahara M, Furukawa T, Ono Y. Int. J. Antimicrob. Agents **2018**; 52:512–514.

P3-1: The Arg165-to-Pro substitution in PmrB in colistin-resistant *Acinetobacter baumannii*

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Emergence of antibiotic resistance, especially the last resort antibiotic, colistin, in *Acinetobacter baumannii* is the most serious problem in healthcare settings [1]. The major mechanism of colistin resistance in *A. baumannii* is the mutation of two-component system sensor histidine kinase, PmrB which upregulates *pmrC* expression and confers resistance to colistin [2].

In our previous study [3], all carbapenem-resistant *A. baumannii* isolates were susceptible to colistin and belonged to the most common sequence types (STs), ST195 and ST542. However, an colistin-resistant *A. baumannii* (L5) was recovered from endotracheal aspirate from a patient at the King Chulalongkorn Memorial Hospital, Bangkok, Thailand in June, 2019. Here, we characterized the *pmrCAB* genes in colistin-resistant *A. baumannii* L5.

A. baumannii L5 was resistant to colistin (MIC=8 mg/L) and belonged to a new ST, ST1953. No mutation of PmrA was found, whereas, the Arg165-to-Pro amino acid substitution in PmrB was found in colistin-resistant *A. baumannii* L5. The expression of *pmrC* in *A. baumannii* L5 was significantly higher than those of colistin-susceptible *A. baumannii*. The presence of Arg165-to-Pro substitution in PmrB was associated with *pmrC* expression and decreased susceptibility to colistin.

[1] J. Nowak, E. Zander, D. Stefanik, PG. Higgins, I. Roca, J. Vila, MJ. McConnell, JM. Cisneros, H. Seifert, MagicBullet Working Group WP4. 2017. High incidence of pandrug-resistant *Acinetobacter baumannii* isolates collected from patients with ventilator-associated pneumonia in Greece, Italy and Spain as part of the MagicBullet clinical trial. *J Antimicrob Chemother*, **72** (2017)

[2] MD. Adams, GC. Nickel, S. Bajaksouzian, H. Lavender, AR. Murthy, MR. Jacobs and RA. Bonomo. Resistance to Colistin in *Acinetobacter baumannii* Associated with Mutations in the PmrAB Two-Component System. *Antimicrob Agents Chemother*, **53** (2009), 3628-3634.

[3] U. Singkham-in and T. Chatsuwan. *In vitro* activities of carbapenems in combination with amikacin, colistin, or fosfomycin against carbapenem-resistant *Acinetobacter baumannii* clinical isolates. *Diag Microbiol Infect Dis*. **91** (2018), 169-174.

[4] S. Gerson, JW. Betts, K. Lucaßen, CS. Nodari, J. Wille, M. Josten, S. Gottig, J. Nowak, D. Stefanik, I. Roca, J. Vila, JM. Cisneros, RM. La Ragione, H. Seifert and PG. Higgins. Investigation of novel *pmrB* and *eptA* mutations in isogenic *Acinetobacter baumannii* isolates associated with colistin resistance and increased virulence *in vivo*. *Antimicrob Agents Chemother*, **63** (2019)

P3-2: Global transcriptional profiles in RND efflux pump mutants

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Acinetobacter baumannii is a Gram-negative opportunistic pathogen which is becoming an increasingly problematic cause of infections owing to its high level of antimicrobial resistance (AMR) associated with high mortality rates [1]. A major contributing factor to AMR is the presence and upregulation of multidrug efflux pumps [2]. Whilst the presence of these efflux pumps is well-established, the regulation, full substrate profile and innate physiological roles of these proteins are yet to be fully elucidated. The aim of this study was to determine the global transcriptomic effect of inactivating the genes encoding for the efflux pumps AdeABC, AdeIJK and AdeFGH from the RND superfamily of efflux pumps.

RNA-Seq transcriptomics was carried out on wild-type AB5075-UW and the efflux pump mutant strains *adeB::tn*, *adeJ::tn* and *adeG::tn* [3] to determine transcriptional profiles. The results of gene cluster analysis indicated the absence of these efflux pumps had downstream effects on iron homeostasis, capsule production and type IV pili gene expression. To determine whether these transcriptional changes were of phenotypic importance, changes in siderophore production, motility and biofilm formation were investigated in the mutant strains compared to the parental strain.

[1] L. C. Antunes, P. Visca and K. J. Towner. *Acinetobacter baumannii*: evolution of a global pathogen. *Pathog. Dis.* **71** (2014), 292-301.

[2] S. Hernando-Amado, P. Blanco, M. Alcalde-Rico, F. Corona, J. A. Reales-Calderon, M. B. Sanchez, and J. L. Martinez. Multidrug efflux pumps as main players in intrinsic and acquired resistance to antimicrobials. *Drug Resist. Updat.* **28** (2016), 13-27.

[3] L. A. Gallagher, E. Ramage, E. J. Weiss, M. Radey, H. S. Hayden, K. G. Held, H. K. Huse, D. V. Zurawski, M. J. Brittnacher and C. Manoil. Resources for Genetic and Genomic Analysis of Emerging Pathogen *Acinetobacter baumannii*. *J. Bac.* **197** (2015), 2027-2035.

P3-3: Unusual occurrence of plasmid-mediated *bla*_{VIM-4} carbapenemase in *Acinetobacter pittii*

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Carbapenem-resistant clinical isolates of *Acinetobacter pittii* are increasingly reported worldwide. Several carbapenem hydrolysing β -lactamases belonging to Amber's class B (NDM-1) or class D (OXA-58-like, OXA-23-like) have been identified in that species [1, 2]. The first characterisation of a VIM-4-producing *A. pittii* strain is reported here.

A. pittii strain 1934 was recovered from a blood culture collected in a patient suffering from haematological disease. According to the CLSI breakpoints, this isolate was resistant to ceftazidime, carbapenems and amikacin, of intermediate susceptibility to piperacillin-tazobactam and was susceptible to cefepime and colistin. PCR experiments showed that this strain carried a class B metallo- β -lactamase encoding gene, which was further identified as *bla*_{VIM-4} by sequencing. Whole genome sequencing (WGS) was performed to fully characterise the resistome of the isolate and the genetic context of *bla*_{VIM-4}. This gene was found to be located on a ca. 274-kb conjugative plasmid, which could be successfully transferred to an *Acinetobacter baumannii* recipient strain. The *bla*_{VIM-4} gene was located in a 3-kb-long integron, itself embedded in a ca 23-kb Tn3-like transposon. Four aminoglycoside resistance genes and two macrolide resistance determinants were also identified on that plasmid. Seventeen insertion sequences were mapped, of which 10 were present in a single copy, two in two copies and one in three copies. According to the Pasteur MLST scheme, strain 1934 belongs to ST119, a genotype already reported to harbour various class B metallo- β -lactamase encoding genes such as *bla*_{NDM-1} and *bla*_{IMP-4}, in various geographical areas [1, 3].

Because *A. pittii* genotype ST119 has a propensity to collect class B metallo- β -lactamases, it represents a potential risk of dissemination of carbapenem resistance to other Gram-negative species, as illustrated here by the plasmid-borne *bla*_{VIM-4} gene.

- [1]. Ang GY, Yu CY, Cheong YM, Yin WF, Chan KG. 2016. Emergence of ST119 *Acinetobacter pittii* co-harbours NDM-1 and OXA-58 in Malaysia. *Int J Antimicrob Agents* 47:168-9.
- [2]. Silva L, Mourao J, Grosso F, Peixe L. 2018. Uncommon carbapenemase-encoding plasmids in the clinically emergent *Acinetobacter pittii*. *J Antimicrob Chemother* 73:52-56.
- [3]. Kamolvit W, Derrington P, Paterson DL, Sidjabat HE. 2015. A case of IMP-4-, OXA-421-, OXA-96-, and CARB-2-producing *Acinetobacter pittii* sequence type 119 in Australia. *J Clin Microbiol* 53:727-30.

P3-4: The novel resistance mechanism of tigecycline in *Acinetobacter baumannii*

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Tigecycline is a potent antibiotic for treating infections with multi-drug resistant *Acinetobacter baumannii* (MDRAB)[1]. With the increasing application of tigecycline, isolates resistant to tigecycline have constantly been reported[2,3,4]. However, the definitive mechanism of tigecycline resistance in MDRAB has not been fully determined. This study aimed to explore the molecular mechanism of tigecycline resistance in *A. baumannii*.

Spontaneous mutants with reduced susceptibility to tigecycline were selected both in liquid and solid medium by serial passage experiments under increasing tigecycline concentration. Five mutations are identified by the whole genome comparison in tigecycline-resistant strains with wild type, and confirmed using PCR and Sanger sequencing, including four point mutations and one insertion. The *adeS* gene was confirmed to cause decreased susceptibility to tigecycline in two ways (*adeS*::ISAb1 and *adeS* E51K) by complementation experiments and antimicrobial susceptibility tests. The *rpoB* gene and the *rrf* gene were also involved in the mechanism of tigecycline resistance in *A. baumannii*. By measuring the relative growth rate, majority of mutants reduced fitness cost with tigecycline and increased fitness cost without tigecycline.

In conclusion, we found that *adeS*, *rrf* and *rpoB* were associated with decreased susceptibility to tigecycline.

[1]Wu, M.T., Chen, H.Y., Ou, T.Y., Kuo, L.N., Cheng, K.J., Lee, W.S., *Clinical evaluation of tigecycline in the treatment of nosocomial infection in a hospital in Taiwan*. Int J Clin Pharmacol Ther, 2014. **52**(12): p. 1030-6.

[2] Anthony, K.B., Fishman, N.O., Linkin, D.R., Gasink, L.B., Edelstein, P.H., Lautenbach, E., *Clinical and microbiological outcomes of serious infections with multidrug-resistant gram-negative organisms treated with tigecycline*. Clin Infect Dis, 2008. **46**(4): p. 567-70.

[3]Wright, M.S., Iovleva, A., Jacobs, M.R., Bonomo, R.A., Adams, M.D., *Genome dynamics of multidrug-resistant Acinetobacter baumannii during infection and treatment*. Genome Med, 2016. **8**(1): p. 26.

[4]Costello, S.E., Gales, A.C., Morfin-Otero, R., Jones, R.N., Castanheira, M., *Mechanisms of Resistance, Clonal Expansion, and Increasing Prevalence of Acinetobacter baumannii Strains Displaying Elevated Tigecycline MIC Values in Latin America*. Microb Drug Resist, 2016. **22**(4): p. 253-8.

P3-5: Detection of active pairs of XerC/D recognition sites mediating fusions and inversions in *Acinetobacter baumannii* plasmids carrying OXA-58 carbapenemase adaptive modules

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Acquired carbapenem-hydrolysing class-D β -lactamases (OXA-type) represent main factors of carbapenem resistance among multi-drug resistant (MDR) *Acinetobacter baumannii* (*Aba*) strains. Similarly to other OXA carbapenemases, OXA-58 genes (*bla*_{OXA-58}) are embedded in plasmid-borne genetic structures which are flanked by a variable number of short 28-nucleotide motifs potentially recognized by the XerC/XerD site-specific recombination system (SSR) of the bacterial hosts (pXerC/D or *pdif* sites) [1]. It is poorly understood, however, how pXerC/D sites mediate mobilisation of the adaptive modules linked to them. We have recently demonstrated in *Aba* strains assigned to CC15 (Pasteur scheme) the existence of an active pair of pXerC/D sites promoting cointegrate formation between plasmids containing a *bla*_{OXA-58}- and *TnaphA6*-resistance structure [1]. Here, we report the presence of additional pairs of pXerC/D sites mediating not only plasmid fusions and resolutions, but also the intra-molecular inversion of the adaptive module.

The sequences of Ab825 plasmids were determined by 454 pyrosequencing and plasmid walking. A 28-nucleotide XerC/D consensus sequence was inferred from a local database of 215 *Aba* plasmids deposited in databases, and used to search potential sites in Ab825 plasmids. Cointegrate formation was detected by transformation of susceptible *Acinetobacter* strains, and the identification of sister pXerC/D pairs active in SSR by PCR and amplicon sequencing.

Aba plasmids of less than 50 kbp were found to contain an average of 3-5 pXerC/D-like sites per molecule, larger plasmids were mostly devoid of these sites. Sequence analysis revealed the presence of three plasmids in Ab825: pAb825_27 (27 kbp), pAb825_12 (12 kbp), and pAb825_9 (9 kbp), each bearing several pXerC/D sites. A co-integrate (pAb825_36) was also detected resulting from the fusion of pAb825_27 and pAb825_9 mediated by an active pair of pXerC/D sites. Two additional active pairs were uncovered, one mediating the intra-molecular inversion of the *bla*_{OXA-58}- and *TnaphA6*-containing structure and the other the fusion between pAb825_27 and pAb825_12. These observations shed light on the role of pXerC/D-mediated SSR in the evolutionary dynamics of *Aba* plasmids and the underlying mechanisms of dissemination of antimicrobial resistance and other adaptive structures among the *Acinetobacter* population.

[1] Cameranesi MM, Morán-Barrio J, Limansky AS, Repizo GD, Viale AM. *Front. Microbiol.* 9 (2018), 66



P3-7: First report of a pan-drug resistant *Acinetobacter baumannii* ST15 recovered from catheter-related bloodstream infection

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Spread of antimicrobial resistant *Acinetobacter baumannii* (ARAB) represents a public health threat and is mainly associated with the dissemination of specific clones [1]. In South America, the clonal complexes CC1, CC15 and CC79 are the main lineages associated with ARAB, particularly with multi- (MDR) or extensively-drug resistant (XDR) [2, 3]. However, detection of isolates presenting pan-drug resistance (PDR), i.e., resistant to all available antimicrobial agents, poses a new and urgent threat to antimicrobial therapy. In this study, we report the genetic determinants of a PDR *A. baumannii* strain determined by whole genome sequencing. The isolate 414.17 was recovered in 2017 from an oncologic patient presenting catheter-related blood stream infection and initially identified by phenotypic and molecular (detection of blaOXA-51-like gene) methods. Antimicrobial susceptibility was determined by Sensititre and in house broth microdilution (for polymyxin B and colistin). Whole genome DNA was sequenced in an Illumina HiSeq platform. Plasmid sizes were analyzed by S1-PFGE and commercial kit (Promega). After quality control of raw data, reads were de novo assembled by using the Spades algorithm with CLC Bench Software. Fasta files were uploaded to ResFinder, CARD and MLST databases. The isolate 414.17 was proven to be non-susceptible to all evaluated antimicrobial agents, including aminoglycosides, quinolones, beta-lactams, colistin, polymyxin and minocycline, and, thus, classified as PDR. MLST analysis identified the isolate belonging to ST15 (Institute Pasteur Scheme) and to a novel allele combination by Bartual Scheme. The following antimicrobial resistance genes (associated with the respective phenotypes) were detected: aac(6')-Iai, aac(3)-Iia, aph(3'')-Ib, aph(6)-Id (aminoglycoside), mutations in gyrA/B and parC/E (fluoroquinolones), blaOXA-51, blaOXA-23, blaTEM-1B, blaADC-25 (beta-lactams), mutations in pmrCAB operon (polymyxins), tet(B) (tetracyclines); the efflux pump genes adeL, adeK, adeN, adeF, adel, abeS were also detected. Three large plasmids of approximately 170kb, 140kb and 10kb were identified by S1-PFGE while a small plasmid of ca. 1.75kb was detected by the commercial kit. To the best of our knowledge, this is the first report of a PDR *A. baumannii* belonging to ST15. Considering the large distribution of this clone in Latin American countries, in which MDR and XDR are considered highly endemic, this finding underscores the urgent need of effective measures to reduce the dissemination of ARAB.

Acknowledgements: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

[1] Diancourt L, Passet V, Nemec A, Dijkshoorn L, Brisse S. The population structure of *Acinetobacter baumannii*: expanding multiresistant clones from an ancestral susceptible genetic pool. PLoS One. 2010;5(4):e10034.

[2] Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268-81.

[3] Rodríguez CH, Nastro M, Famiglietti A. Carbapenemases in *Acinetobacter baumannii*. Review of their dissemination in Latin America. Rev Argent Microbiol. 2018;50(3):327-333.

P3-8: Emergence of polymyxin resistance among clinical isolates of multi-drug resistant *Acinetobacter baumannii* ST25 from Brazil

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Spread of antimicrobial-resistant *Acinetobacter baumannii* (ARAB) represents a public health threat and is mainly associated with the dissemination of specific clones [1]. In South America, the clonal complexes CC1, CC15 and CC79, and, in a less extent, CC25, are the main lineages associated with ARAB, particularly with multi- (MDR) or extensively-drug resistant (XDR) [2, 3]. Nevertheless, isolates of *A. baumannii* belonging to CC25 have consistently been detected in Brazil, including isolates harboring *bla*NDM-1 gene. The aim of the present study is to describe the occurrence of polymyxin resistance among clinical isolates of multi-drug resistant *Acinetobacter baumannii* ST25 from different hospitals in Brazil. A total of 134 isolates of ARAB were subjected to *bla*OXA-genes detection and prediction of clonal complexes by a modified 3-loci sequencing typing [4]. Antimicrobial susceptibility was determined by Sensititre and in-house broth microdilution (for polymyxin B). Whole genome DNA was sequenced in an Illumina HiSeq platform. After quality control of raw data, reads were de novo assembled by using the Spades algorithm with CLC Bench Software. Fasta files were uploaded to CARD and MLST databases. A total of 12 (9%) isolates were identified as ST25 (Institute Pasteur Scheme), and included in this analyses. All the isolates were classified as MDR (multi-drug resistant) according to international criteria [2]. Polymyxin resistance was detected in 4/12 isolates (33%) (MIC \geq 4 ug/mL) with confirmation by in-house broth microdilution. According to the CARD database, all isolates harbored the *bla*OXA-64 gene (a *bla*OXA-51-like allele), belonging to International Group 4. Besides, *bla*OXA-23 were detected in 10 isolates (83%), *bla*OXA-72 in 3 isolates (25%), and *bla*OXA-253 in 1 isolate (8%). Co-occurrence of *bla*OXA-23 and *bla*OXA-72 was detected in 3 isolates (25%). All the 4 isolates presenting polymyxin resistance were positive for the *bla*OXA-23 gene. Our results highlight the diversity of oxacillinases among MDR ST25 *A. baumannii*, and alert for the emergence of polymyxin resistance among emerging clones in Brazil.

Acknowledgements: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

[1] Diancourt L, Passet V, Nemec A, Dijkshoorn L, Brisse S. The population structure of *Acinetobacter baumannii*: expanding multiresistant clones from an ancestral susceptible genetic pool. PLoS One. 2010;5(4):e10034.

[2] Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268-81.

[3] Rodríguez CH, Nastro M, Famiglietti A. Carbapenemases in *Acinetobacter baumannii*. Review of their dissemination in Latin America. Rev Argent Microbiol. 2018;50(3):327-333.

[4] Martins N, Picão RC, Cerqueira-Alves M, Uehara A, Barbosa LC, Riley LW, Moreira BM. A new trilocus sequence-based multiplex-PCR to detect major *Acinetobacter baumannii* clones. Infect Genet Evol. 2016 Aug;42:41-5.

P3-9: Plasmid content of a clinical IC4 *Acinetobacter baumannii* isolate from Bolivia

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Acinetobacter baumannii is a multidrug-resistant nosocomial pathogen, capable of causing serious infections. Acquisition of antimicrobial resistance genes is often plasmid-encoded, but very few data is available from Bolivia. The objective was to characterise the plasmid content of an IC4 *A. baumannii* isolate from a Bolivian clinical sample.

The isolate (MC75) was recovered from an ulcer in an 84-year-old man. MICs for ciprofloxacin, gentamicin, imipenem, meropenem, and tigecycline were determined by agar dilution, and for colistin by broth microdilution, using EUCAST breakpoints. A MiSeq platform was used for whole genome sequencing. Resistome, STs (Ox/Pas) and core genome MLST were obtained using *de novo* assemblies. Plasmid characterisation was performed by S1-PFGE and Southern blot using digoxigenin-labelled probes for *bla*_{OXA-23-like}, *strA* and *bla*_{OXA-51-like}. Plasmid assemblies were confirmed using PCR-based gap closure and MinION sequencing. Conjugation experiments were carried out with the recipient strains *A. baumannii* BM4547 and *Escherichia coli* J53 and transconjugants were screened by PCR.

cgMLST revealed that MC75 belonged to IC4 and was ST236^{Ox}/15^{Pas}. It was resistant to all antimicrobials except colistin and tigecycline. The carbapenemase *bla*_{OXA-23} was encoded on the chromosome in a Tn2008 structure while *strA* was encoded on a 150Kb plasmid (pMC75.1), also harboring *strB* and *sul2* in a Tn6172 and a mercury resistance operon. The plasmid also encoded for the genes of the BREX type 1 system that has been described to be involved in phage resistance. Its scaffold was similar to pMC1.1 and pD46-4. MC75 also carried a 13Kb plasmid (pMC75.2) harboring *bla*_{TEM-1B}, *aac(3)-IIa*, septicolysin, a TonB-dependent receptor and a toxin-antitoxin system and *mob* genes that are related to conjugation. pMC75.2 was able to conjugate to BM4547 but not to J53.

Two plasmids encoding multiple antibiotic resistance genes were found in this IC4 clinical isolate as well as Tn2008 encoding carbapenem resistance on the chromosome. All the antimicrobial resistance genes were found to be associated with IS elements, constituting transposons that lead to their mobilisation and make genetic rearrangements more likely to happen. In addition, the accumulation of antimicrobial resistance determinants in mobile genetic elements, especially conjugative plasmids, is of great concern because of the potential to spread to other isolates and further limiting antimicrobial treatment options.

P3-10: Investigation of the contribution of a putative mobilization gene to the dissemination of an OXA-231 encoding plasmid in *Acinetobacter baumannii*

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Background: Conjugative or mobilizable plasmids are one of the most important drivers of antimicrobial resistance in bacteria. The present study aimed to characterise OXA-231, an OXA-143-like encoding plasmid, and the contribution of a putative mobilization gene to the dissemination of the carbapenemase encoding plasmid in a clinical *Acinetobacter baumannii* isolate.

Materials/methods: The *A. baumannii* isolate AF81 recovered from Brazil in 2008 was analysed. Whole genome sequencing was performed on a MiSeq and a MinION platform. Hybrid- and non-hybrid methods were used for *de novo* assembly and to determine seven-loci MLST (Oxford typing scheme). Plasmid DNA was extracted and used for electroporation with *A. baumannii* ATCC 17978 and transformants were selected using ticarcillin. The wt pAF81 encoding a putative mobilization gene was eliminated from the AF81 strain. Using a PCR-mediated deletion of the putative mobilization gene encoded on the OXA-143-like pAF81, pAF81- Δ mob was generated. Finally, pAF81- Δ mob was introduced to the pAF81 deficient AF81 isolate by electroporation. Conjugation experiments were carried out using the rifampicin resistant *A. baumannii* BM4547 isolate as a recipient. Transconjugants were tested by PCR for the presence of OXA-143-like encoding plasmid and susceptibility to meropenem by disc diffusion.

Results: The AF81 isolate from Brazil was ST1552 and did not cluster with any international clone (IC). The isolate harboured a plasmid with a size of 3.955 bp carrying a GR19 replicase. The plasmid encoded *bla*_{OXA-231} (a *bla*_{OXA-143} variant differing by one amino acid substitution from OXA-143) and harboured *mob*, a putative mobilization gene. The *mob* gene contained a conserved domain of the Mob/Pre protein family, recombinases involved in plasmid mobilization. Electroporation and conjugation experiments demonstrated the transferability of the OXA-143-like plasmid. Conjugation experiments with the AF81 isolate harbouring the pAF81- Δ mob resulted in the transfer of the plasmid despite the deletion of the putative mobilization gene.

Conclusions: The present study describes a conjugative plasmid involved in the dissemination of the OXA-143-like carbapenemase. These data demonstrated that the putative *mob* gene harboured by the OXA-143-like encoding plasmid was not essential for the mobilization of the plasmid, and therefore another mobilization mechanism must be associated with its dissemination.

P3-11: Association of overexpression of *abeM* efflux pump gene with antibiotic resistance in *Acinetobacter baumannii* strains clinically isolated from a tertiary hospital

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A. baumannii is the most frequently implicated in nosocomial infections [1]. Recently, isolates of XDR *A. baumannii* have accounted for more than 30% of clinical isolates in Korean hospitals [2]. This study investigated the prevalence of extended spectrum β -lactamases (ESBLs) genes and relative expression levels of efflux pump genes in XDR *A. baumannii* clinically isolated from a Korean tertiary hospital.

A total of 40 *A. baumannii* (20 XDR and 20 wild type) were isolated from a university hospital in Korea. We extracted the DNA and RNA from all isolates, and performed PCR for ESBLs genes, including *blaKPC*, *blaIMP*, *blaVIM*, *blaSPM*, *blaGIM*, *blaSIM*, *blaOXA-23*, *blaOXA-24*, *blaOXA-51*, *blaOXA-58*. Expression levels of seven efflux pump genes, *adeB*, *adeJ*, *adeE*, *adeG*, *abeM*, *craA*, *amvA* were measured by real-time reverse-transcription PCR.

Class D β -lactamase genes, *blaOXA-23* and *blaOXA-51* were presented in all 20 XDR isolates, and *blaOXA-51* was presented in 7 (35%) wild type isolates. Other ESBL genes were completely absent in all isolates. Relative expression levels of *adeB*, *adeJ*, *adeG*, *abeM* genes in XDR isolates were more 10-fold than those in wild type isolates. Especially, we found a significant association between the increased expression of *abeM* and XDR isolates. Also, *adeB* gene showed close relation to XDR isolates with Odds ratio 2.73.

We demonstrated that clinically isolated XDR *A. baumannii* carry *blaOXA-23* and *blaOXA-51* genes and overexpress efflux pump AdeABC, AdeIJK, AdeFGH, AbeM, and CraA. We also found *blaOXA-23* gene was detected exclusively in XDR isolates and *abeM* gene showed largest relative expression level. In conclusion, OXA-23 carbapenemase and AbeM efflux pump may significantly be associated with XDR *A. baumannii*.

[1] Coyne S, Courvalin P, Périchon B. Efflux-Mediated Antibiotic Resistance in *Acinetobacter* spp. *Antimicrob Agents and Chemother.* **55**(2011),947–953

[2] Wieczorek P, Sacha P, Hauschild T, Zórawski M, Krawczyk M, Tryniszewska E. Multidrug resistant *Acinetobacter baumannii*-the role of AdeABC (RND family) efflux pump in resistance to antibiotics. *Folia Histochem Cytobiol.* **46**(2008), 257–267

P3-12: Novel *pmrB* mutations of laboratory-evolved and clinical *Acinetobacter baumannii* isolates

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Acinetobacter baumannii evolved multiple mechanisms of resistance to polymyxin B, the one of mechanisms being the modification or removal of the phosphate groups on lipid A, thereby reducing the net negative charge of the outer membrane surface [1]. This process is regulated by *pmrAB* two-component regulatory systems (TCS) [2]. Through whole genome sequencing (WGS) of laboratory-evolved PMR^{Low} and PMR^{High} strains, we discovered common mutations in genes including *lpp* and the sensor kinase *pmrB*. Duplication of the *relBE* antitoxin-toxin system occurred only in PMR^{High}. Point mutations in *pmrB* have been widely observed in laboratory-evolved polymyxin B-resistant strains and multidrug-resistant clinical isolates of *Acinetobacter baumannii*. In our studies, Point mutations of *pmrB* were observed in PMR^{High} (polymyxin B MIC > 16 µg/ml) and multidrug-resistant clinical isolates (polymyxin B MIC range 2 to 128 µg/ml). The histidine kinase domain was mutated in all of 10 PMR^{High} strains (T235I, A236E, R263S). The HAMP domain-containing histidine kinase was mutated in polymyxin B-resistant clinical isolate F-1629 (P170L). These point mutations in the histidine kinase may lead to polymyxin B resistance by maintaining phosphorylation or inducing autophosphorylation of transcriptional regulator *pmrA*.

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- [1] Matthew J. Powersa, and M. Stephen Trent: Phospholipid retention in the absence of asymmetry strengthens the outer membrane permeability barrier to last-resort antibiotics. *PNAS*. **115** (2018)
- [2] Luis A. Arroyo, Carmen M. Herrera, Lucia Fernandez, Jessica V. Hankins, M. Stephen Trent, and Robert E. W. Hancock: The *pmrCAB* Operon Mediates Polymyxin Resistance in *Acinetobacter baumannii* ATCC 17978 and Clinical Isolates through Phosphoethanolamine Modification of Lipid A. *AAC*. **55** (2011)

P3-13: Overproduction of outer membrane vesicles in laboratory-evolved *Acinetobacter baumannii*

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Outer membrane vesicles (OMVs) containing various bacterial compounds are released from mainly gram-negative bacteria. Secreted OMVs play important roles in the ability of a bacterium to defend itself, and thus contribute to the survival of bacteria in a community. But, mechanisms of OMVs to resist cationic antimicrobial peptides called polymyxin B (PMB) in *Acinetobacter baumannii* are still unclear. In this study, we constructed laboratory-evolved PMB-resistant strains including PMR^{Low} and PMR^{High}. When the amount of OMVs production was measured using lipophilic fluorescence dye FM4-64 and fluorescence-activated cell sorting (FACS), PMR^{High} produced OMVs significantly high compared to antibiotic susceptible strains. Interestingly, transmission electron microscopy revealed that OMVs were formed on the surface membrane of PMR^{High} not on Lab-WT. Zeta-potential analysis proved that membrane-negative charge decreased in PMR-resistant strains, resulting in resistance to PMB possibly by reduction of PMB binding to cell surface. We also investigated OMVs from resistant strains could protect susceptible cells from PMB-induced cell death. Taken together, present study demonstrates that OMVs from PMB-resistant *A. baumannii* play important roles as a protective effect under PMB condition.

This work was supported by grants from the National Research Foundation of Korea (No. NRF-2017R1A2B4005838).

- [1] Carmen Schwechheimer and Meta J. Kuehn. Outer-membrane vesicles from Gram-negative bacteria: biogenesis and functions. *Nat* **13** (2015), 605-619
- [2] Si Won Kim, Seong Bin Park and Tae Sung Jung. Outer membrane vesicles from β -lactam-resistant *Escherichia coli* enable the survival of β -lactamsusceptible *E. coli* in the presence of β -lactam antibiotics *SCIENTIFIC REPORTS*. **5** (2018), 939-951.
- [3] Rachel L. Soon, Roger L. Nation and and Jian Li, Different surface charge of colistin-susceptible and -resistant *Acinetobacter baumannii* cells measured with zeta potential as a function of growth phase and colistin treatment. *J Antimicrob Chemother* 2011; **66**: 126–133
- [4] Benjamin I. Barrda, Ryszard A. Zielke, Adriana Le Van and E. Sikora. *Neisseria gonorrhoeae* MlaA influences gonococcal virulence and membrane vesicle production. *PLoS Pathog*, **15** (2019)
- [5] M. Gebhardt, L. Gallagher, R. Jacobson, E. Usacheva, L. Peterson, D. Zurawski and H. Shuman. Joint transcriptional control of virulence and resistance to antibiotic and environmental stress in *Acinetobacter baumannii*. *mbio* **6** (2015)

P3-14: Colistin resistance in *Acinetobacter baumannii* is associated with the StkR two-component system

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Multidrug resistant bacterial infections are increasing in frequency worldwide. Subsequently, the reduced efficacy of drugs places enormous pressure on treatment regimes and as such increasing our understanding of how these pathogens evade treatment is urgently required. *Acinetobacter baumannii* is a major Gram-negative opportunistic hospital-acquired pathogen that can cause a range of severe infections in humans. *A. baumannii* can colonise any number of anatomical sites potentially leading to acute or chronic disease. The mechanisms by which this bacterium is able to cause disease are multifactorial and are likely to be under stringent genetic control. To provide insight into these regulatory networks within *A. baumannii* we examined the role of a response regulatory element within the multidrug resistant Australian clinical isolate *A. baumannii* 04117201. The response regulator protein is part of a two-component system that works in concert with a membrane-bound histidine kinase to respond to external signals. The response regulator encoded by *stkR* of the two component system *stkRS* was deleted using site-specific recombination. The resulting Δ *stkR* deletion strain was assessed for alterations in bacterial cell adherence, surface hydrophobicity, and its resistance profile to selected antibiotics including colistin, known as a “last resort” drug. Compared to the parent, the Δ *stkR* strain demonstrated a 2-fold increase in eukaryotic cell adherence, increased cell surface hydrophobicity and a 2-fold increase in colistin resistance. Protein analysis of the Δ *stkR* strain when grown in the presence of increasing concentrations of colistin identified proteins that were differentially expressed compared to the parent strain. Previous studies have shown that colistin resistance in *A. baumannii* strains can be due to major alterations in lipid A in the outer membrane. However, examination of the surface polysaccharides of the *A. baumannii* 04117201 Δ *stkR* strain revealed only a minor change in this region. Analysis of RNA sequencing data identified a number of genes that were differentially expressed and has potentially identified the source of the increased colistin resistance, representing a novel mechanism of resistance in *A. baumannii* to this important antibiotic.

P3-15: Investigating the contribution of RND-type efflux pump AdeABC to carbapenem resistance in *Acinetobacter baumannii* ATCC 19606

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Objectives: Carbapenem resistance is common in *Acinetobacter baumannii* and is mostly mediated by oxacillinases. Some literature suggests that the RND-type efflux pump AdeABC also contributes to carbapenem resistance [1,2,3].

AdeABC is regulated by the 2-component regulator AdeRS. Mutations or disruption of these genes have been shown to affect *adeABC* expression [4]. We have previously shown that *A. baumannii* ATCC 19606 *adeRS* knockout (Δ *adeRS*) does not express *adeABC* and reveals increased susceptibility to aminoglycosides, macrolides, fluoroquinolones, chloramphenicol, rifampicin, tetracyclines and glycylicyclines compared to ATCC 19606 wildtype (wt).

We sought to investigate the contribution of AdeABC to carbapenem resistance when combined with OXA carbapenemases in ATCC 19606.

Methods: Transformation of Δ *adeRS* and wt strain was performed with the shuttle plasmid pWH1266 containing either the carbapenemase-encoding genes *bla*_{OXA-40} or *bla*_{OXA-143}, or with the *bla*_{OXA-231}-containing plasmid pAF81 obtained from a clinical isolate.

Minimal inhibitory concentrations (MICs) for imipenem (IPM) and meropenem (MEM) were determined using agar dilution.

Results: Agar dilution revealed that ATCC 19606 wt and Δ *adeRS* were susceptible to MEM and IPM. Deletion of *adeRS* had no impact on IPM susceptibility (MIC: 0.5 mg/L), whereas it reduced the MIC for MEM by one dilution step (wt: MIC: 0.5 mg/L; Δ *adeRS*: MIC: 0.25 mg/L).

Transformation with plasmids containing *bla*_{OXA-40}, *bla*_{OXA-143} or *bla*_{OXA-231} caused resistance to MEM and IPM in both Δ *adeRS* strain and its parental strain. Furthermore, knock-out of *adeRS* led to no change or one dilution lower MIC compared to the parental strain.

Conclusion: This study shows that the contribution of the efflux pump AdeABC to carbapenem susceptibility is negligible in either wildtype or strains transformed with a carbapenemase.

1 Huang *et al.* Diagn Microbiol Infect Dis. 2008; 62:326-32

2 Lee *et al.* Ann Clin Lab Sci. 2010; 40:43-48

3 Zhang *et al.* Infect Drug Resist. 2018; 11:457-467

4 Gerson *et al.* J Antimicrob Chemother. 2018; 73:1501-1508

P3-16: Resistance to sulbactam-durlobactam in clinical isolates of *Acinetobacter baumannii* is rare and maps to PBP3

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Effective treatments for infections caused by *Acinetobacter baumannii* (*Ab*) are desperately needed. These infections are considered a significant public health concern due to high mortality rates associated with multidrug resistance. Historically, sulbactam (SUL), a class A β -lactamase inhibitor (BLI), could be used for the treatment of these infections due to its intrinsic antibacterial activity against *Ab*. However, its therapeutic utility has been severely compromised due to the emergence of resistance. We previously showed that laboratory-derived SUL resistance mapped to residues proximal to the active site of PBP3 at very low frequencies, and were associated with a fitness cost [1]. Durlobactam (DUR, previously known as ETX2514), a broad-spectrum inhibitor of Class A, C and D serine β -lactamases [1], is currently in Phase 3 clinical testing in combination with SUL for the treatment of carbapenem-resistant *Ab*. Multiple, unbiased, global surveillance studies have shown that DUR restores SUL susceptibility (MIC \leq 4 mg/L) in 99% of clinical isolates (>3,600 tested to date). 59% of these were found to be carbapenem-non susceptible (CARB-NS). Whole genome sequencing analyses revealed that the rare SUL-DUR-resistant *Ab* strains encoded either A515V, T526S or I343F variants of PBP3 [3]. These mutant alleles were cloned, purified and compared to wildtype PBP3 for relative binding to SUL and other β -lactams (BLs). The T526S mutant showed an almost complete loss of SUL binding, whereas A515V and I343F mutants showed only ~2-fold reduction. All three mutant PBP3s had a significant reduction in imipenem binding. Meropenem binding to PBP3 was lowered by A515V and T526S but not I343F. Aztreonam binding was modestly affected in all three mutants. Taken together, these results show that: (1) the vast majority (99%) of SUL resistance in *Ab* is serine β -lactamase-mediated, which can be mitigated by combining with DUR and (2) specific mutations in PBP3, while very rare, can confer resistance to SUL-DUR. This is in contrast with recently approved BL-BLIs such as AvyCaz and Zerbaxa, where pre-existing clinical resistance was reported to be as high as 20% in certain target pathogens, such as CARB-NS *P. aeruginosa* [4].

[1] Penwell, *et al.*, (2015) *Antimicrob. Ag. Chemother.* 59: 1680-89.

[2] Durand-Reville, *et al.*, (2017) *Nature Microbiol.* 2: 17104.

[3] McLeod, *et al.*, (2019) Poster 1185, ECCMID, Amsterdam.

[4] van Duin & Bonomo (2016) *Clin. Infect. Dis.* 63:234 – 241.

P3-17: Inhibition of the RND-type efflux pump regulator AdeRS in *Acinetobacter baumannii*

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Background: Efflux pumps can contribute to reduced susceptibility to numerous antimicrobials. In *A. baumannii* the most important resistance-associated efflux pumps belong to the resistance-nodulation-cell division (RND) efflux pump family [1]. The use of efflux pumps inhibiting antimicrobials in clinical practice is difficult due to their toxic nature against eukaryotic efflux pumps. Therefore, addressing the regulators of bacterial efflux systems has the potential to be a worthwhile alternative to overcome efflux mediated antimicrobial resistance [2]. The RND efflux pump AdeABC is regulated by the 2-component regulator AdeRS and mutations or disruption of the regulators has been shown to affect *adeABC* expression [3]. The objective of this study was to determine the impact of AdeRS inhibition on the antimicrobial resistance phenotype of *A. baumannii*.

Materials/methods: Inhibition of the AdeRS regulator system was introduced into *A. baumannii* ATCC 17978 and 19606 by genetic knockout using markerless mutagenesis [4]. Minimal inhibitory concentrations (MICs) for aminoglycosides, macrolides, fluoroquinolones, carbapenems, chloramphenicol, rifampicin, tetracyclines and glycylicyclines were determined using agar dilution. Gene expression was determined using endpoint RT-PCR.

Results: Whereas deletion of *adeRS* caused increased susceptibility to the tested aminoglycosides, macrolides, fluoroquinolones, carbapenems, tetracyclines and glycylicyclines in ATCC 19606, loss of the regulator system in ATCC 17978 had no significant effect on susceptibility to the tested substances. Endpoint RT-PCR revealed significantly reduced expression of *adeABC* in the *adeRS* knockout of ATCC 19606 compared to its parental strain. On the other hand, ATCC 17978 had low *adeABC* expression in the wild type, suggesting that it is a cryptic gene in this strain.

Conclusions: This study shows that the RND-type efflux pump regulators AdeRS contribute more to the antimicrobial susceptibility phenotype in ATCC 19606 than in ATCC 17978. Furthermore, our results suggest that addressing efflux pump regulators is a possibility in isolates that overexpress AdeABC and can lead to a significant reduction in MICs to a battery of antimicrobials.

[1] Nikaido *et al.* Fems Microbiology Reviews 2012, 36: 340-363

[2] Lomovskaya *et al.* Biochemical Pharmacology 2006, 71: 910-918

[3] Gerson *et al.* Journal of Antimicrobial Chemotherapy 2018, 73: 1501-1508

[4] Stahl *et al.* Plos One 2015, 10: e0138360

P3-18: The role of *adeRS* in reduced antimicrobial susceptibility during motility in *Acinetobacter baumannii* ATCC 17978

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Background: In *Acinetobacter baumannii* the resistance-nodulation-cell division (RND) efflux pump AdeABC, which is regulated by AdeRS, is associated with reduced antimicrobial susceptibility. We previously demonstrated that an *adeRS* knockout in the non-motile *A. baumannii* ATCC 19606 did not express *adeABC* and revealed an increased susceptibility to antimicrobials of different classes.

A. baumannii ATCC 17978 is motile. A β -galactosidase reporter assay revealed that ATCC 17978 did not express *adeRS* and *adeABC* when it is motile. Furthermore, we found that motility was abolished at subinhibitory antimicrobial concentrations.

Based on these observations, we hypothesise a causality between *adeRSABC* inhibition and reduced antimicrobial susceptibility during motility.

Materials/methods: An ATCC 17978 *adeRS* knockout was created by markerless mutagenesis. Antimicrobial susceptibility was determined using a modified agar dilution method using motility plates (0.5% agarose, 5 g/L tryptone and 2.5 g/L NaCl). Minimal inhibitory concentrations (MICs) and minimal motility inhibitory concentrations (MMICs; the concentration where motility is inhibited) for azithromycin, ciprofloxacin, gentamicin, meropenem, tetracycline and tigecycline were determined.

Results: ATCC 17978 *adeRS* knockout and its parental strain exhibited a motile and a non-motile phenotype depending upon the antimicrobial concentration. Motility was observed until a concentration was reached that inhibited motility which we termed the MMIC. Thereafter, the cells grew in the inoculation zone, but no longer spread from this point. MICs were generally higher than the observed MMIC. The MICs and the MMICs differed by only a twofold dilution for tetracycline and tigecycline, whereas a fourfold dilution difference was found for gentamicin and meropenem, an eightfold dilution difference for ciprofloxacin and a sixteen-fold dilution difference for azithromycin. Deletion of *adeRS* had no impact on MICs or MMICs for the tested antimicrobials.

Conclusions: The hypothesis that AdeRS inhibition in motile ATCC 17978 causes reduced antimicrobial susceptibility was disproven with the present results. Therefore, the mechanism, which causes increased antimicrobial susceptibility in motile *A. baumannii*, has to be further investigated.

P3-19: The MFS-type transporters (CraA and TetA) of *Acinetobacter baumannii* AYE

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Acinetobacter baumannii is a worldwide opportunistic pathogen responsible for nosocomial infections. One of the main factors contributing to multidrug resistance in *A. baumannii* is the up-regulation of various efflux pumps, which expel toxic compounds out of the cells with high efficiency [1]. CraA, a member of the Major Facilitator Superfamily (MFS) is a homolog of *E. coli* multidrug efflux transporter MdfA, but was reported to confer resistance in *A. baumannii* against chloramphenicol only [3]. The tetracycline transporter TetA is the major tetracycline-resistance determinant. In *A. baumannii* AYE, *tetA* is located on the Tn1721 like-transposon of AbaR1 resistance island [4].

CraA was heterologously overproduced in *E. coli* and its substrate specificity was determined by drug susceptibility assays and whole cell fluorescent dye uptake experiments. We observed that *craA* overexpression in *E. coli* resulted in multidrug resistance towards phenicols, monovalent cationic compounds (ethidium and TPP+), antiseptics (chlorhexidine and dequalinium), a biocide (benzalkonium), and a chemotherapeutic agent (mitomycin C). We showed that CraA is a drug/H⁺ antiporter by ACMA quenching in everted CraA containing membrane vesicles. Differential gene analysis via RT-qPCR confirmed that *craA* was significantly up-regulated in *A. baumannii* exposed to chloramphenicol.

E. coli overexpressing *tetA* confers resistance towards tetracycline, minocycline, doxycycline, and tigecycline. We also observed that *A. baumannii* WT and $\Delta adeIJ$ complemented with plasmid-encoded *tetA* are significantly less susceptible to tigecycline, compared to the non-complemented strains. Differential gene expression of *tetA* and Resistance Nodulation cell division (RND) efflux pumps (*adeB*, *adeG*, *adeJ*) showed that *tetA* and *adeG* were significantly up-regulated in *A. baumannii* AYE exposed to tigecycline.

We conclude that *craA* encodes a broad-spectrum efflux pump rather than a specific chloramphenicol transporter. TetA confers resistance to several tetracyclines, including tigecycline which is a last-resort antibiotic. MFS-type single-component transporters like CraA and TetA play an important role in antibiotic resistance by removing the drug from cytoplasm to periplasm, from where subsequently the RND-type transporters extrude the drugs across the outer membrane. The synergy between these two classes of transporter is essential for *A. baumannii* to confer efflux-mediated drug resistance.

[1] Yoon E-J et al (2015) Contribution of resistance-nodulation-cell division efflux systems to antibiotic resistance and biofilm formation in *Acinetobacter baumannii*. mBio; 6: e00309-15.

[2] Roca I et al (2009) CraA, a major facilitator superfamily efflux pump associated with chloramphenicol resistance in *Acinetobacter baumannii*. Antimicrob Agents Chemoth; 53: 4013-4.

[3] Edgar R, Bibi E (1997) MdfA, an *Escherichia coli* multidrug resistance protein with an extraordinarily broad spectrum of drug recognition. J Bacteriol; 79: 2274–80.

[4] Fournier PE et al (2006) Comparative genomics of multidrug resistance in *Acinetobacter baumannii*. PLoS Genet; 2: e7

P3-20: Experimental evolution and genomics of antibiotic resistance in *Acinetobacter baumannii*

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Rapid spread of antibiotic resistance, especially in difficult-to-treat gram-negative pathogens like *Acinetobacter baumannii*, calls for systematic efforts to develop more “irresistible” drugs. To gain deeper understanding of dynamics and mechanisms of antibiotic resistance acquisition by *A. baumannii*, we have established the approach combining experimental evolution with time-resolved deep sequencing of evolving microbial populations. This approach includes: (i) competitive outgrowth of replicate cultures with increasing antibiotic concentrations in a custom-engineered morbidostat device; (ii) collection of populations samples in time-series and whole-genome sequencing at ~1,000-fold coverage; (iii) quantitative, dynamic and functional assessment of emerging genetic variations; and (iv) characterization of representative clones to connect genotypes (specific mutations and genome rearrangements) with phenotypes (acquired resistance and fitness).

This approach and typical results will be illustrated on the example of the broadly used antibiotic ciprofloxacin. The observed two-stage evolutionary trajectory includes: (i) highly specific single amino acid substitutions in the primary DNA gyrase target (GyrA) rendering it less sensitive to ciprofloxacin inhibition without appreciable loss of fitness, followed by (ii) additional mutations affecting secondary target genes (*parC*, *parE*) and/or transcriptional regulation of efflux transporters. This evolutionary strategy of *A. baumannii*, while being generally similar to one observed for *E. coli* under comparable experimental conditions, reveals characteristic differences, primarily in dynamics (higher overall frequency of mutations) and more prominent contribution of mobile elements and genome rearrangements (e.g. for inactivation of efflux pump repressor AdeN).

An important practical application of the developed approach is for ranking of different antibiotics by dynamics of resistance acquisition under computer-controlled standard conditions. Thus, we have successfully applied this approach for benchmarking and prioritization of novel drug candidates targeting *A. baumannii*.

P3-21: War, AntiMicrobial Resistance, and *Acinetobacter baumannii* (WAMRA)

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Wars have been a great burden on humanity for a long period of time. The high medical costs of wars particularly those incurred in treating patients with multi-drug resistant infections is probably one of its most adverse effects. *Acinetobacter baumannii* is one of those superbugs which has gained much notoriety during times of wars for causing multi-drug resistant infections among injured military and civilian personnel. The rapidly evolving resistance of this bacterium particularly during this incidence hints out the role of bacterial milieu in promoting the emergence of this highly resistant pathogen. Since military regions are considered hot spots for heavy metals contamination, we hypothesize that exposure of *A. baumannii* to heavy metals coming from shelling and use of ammunitions in war regions might be correlated with its increased levels of antimicrobial resistance (AMR). Therefore, herein we aim to investigate the effects of heavy metals on AMR of *A. baumannii* clinical isolates particularly those originating from war patients and determine the mechanisms implicated at the molecular level.

BMD susceptibility assay showed a wide range of resistance to almost all classes of antimicrobial agents in all tested isolates. Moreover, multi-heavy metal resistance phenotypes were observed in all isolates from both war injuries and non-war injuries. The antimicrobial susceptibility patterns obtained in combination testing showed potential positive association between some heavy metal ions and antimicrobial resistance. Through induction of resistance and WGS, heavy metals such as Copper, Cobalt, Zinc, and Cadmium were shown to have co-selection potential for Cefepime resistance. Lead was shown to have co-selection potential for Gentamicin resistance while Arsenate was shown to possess co-selection potential for Colistin resistance. WGS on "Arsenic and Gentamicin" mutant revealed a potential novel resistance mechanism to Arsenate which is reduced uptake through phosphate transporters.

This is the first study to describe the clinical impact of heavy metals use in military weapons on antimicrobial resistance of *A. baumannii*. This study has helped us understand better the mechanisms of emergence of antimicrobial resistance in bacteria and it has reaffirmed the hypothesis that heavy metal ions are potential and potent drivers of antimicrobial resistance. Most importantly, our study highlights that there is very high risk of co-selection of heavy metal and antimicrobial resistance to occur in war regions given the high concentrations of heavy metals in these regions which exceed significantly the concentrations we used to raise resistance in vitro. It also calls for further research to better understand the mechanisms of co-selection by heavy metals. In addition, it prompts health organizations and policy makers to spread awareness and issue stringent legislations to reduce heavy metals contamination in the environment for effectively combating AMR.

P3-22: Curing of large genomic islands AbaR in *A. baumannii*

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AbaR islands are potential contributor to multi-drug resistance of *A. baumannii*, with a great diversity in gene content and carrying multiple putative antibiotic-resistance genes. However, their effective contribution to antibiotic resistance remains elusive. In a previous study, we identified two *A. baumannii* GCI strains highly naturally transformable[1]. To gain insight into the contribution of AbaR to *A. baumannii* antibiotic resistance, we exploited natural transformation to remove AbaR islands. The two *A. baumannii* strains used were the epidemic and nosocomial strain AYE (France) and the strain AB5075 isolated from a bone infection (military hospital, USA). Both carry AbaRs island of respectively 86 kb and 19.6 kb-long. Using natural transformation and PCR assembly, the AbaR islands were replaced by genetic recombination with a cassette bearing a selection marker (*aac*, resistance to apramycin) and a counter-selection marker (*sacB*, sensitivity to sucrose). Then transformation was carried out with an assembly PCR carrying the insertion site cured of AbaR followed by genetic analysis of sucrose-resistant and apramycin-sensitive clones. Antibiotic susceptibilities were then compared between the parental strains and their AbaR-cured derivatives (disk diffusion tests and E-tests).

The AbaR of AB5075 contains 19 ORFs with seven predicted to confer metal resistance, three of unknown function and one (*sup*) related to antibiotic resistance (sulfate permease). As anticipated, the resistance profiles to 17 antibiotics were identical between the wild-type and the AbaR-cured strain AB5075. In contrast, the AbaR of AYE, carries 25 ORFs with a predicted association with various classes of antibiotics. Accordingly, the AbaR of AYE confers resistance to multiple antibiotics: Aminoglycosides (Amikacin, Gentamicin, Tobramycin), Fosfomycin, Tetracycline, Fluoroquinolone (Ciprofloxacin), Piperacillin, Ticarcillin, Cephalosporines (Ceftazidime, Cefepime) and Monobactam (Aztreonam). Thus, contrarily to AB5075, curing the 86 kb-long AbaR of AYE has a drastic effect on antibiotic resistance profile. We did not observe a paradoxical role of AbaRs regarding fluoroquinolones [2].

By exploiting natural transformation, we setup a genetic system to cure large genomic islands from two MDR clinical strains of *A. baumannii*. Moving beyond predicted resistance, this system allows experimental determination of the antibiotic resistance conferred by the island.

[1] A.-S. Godeux, A. Lupo, M. Haenni, S. Guette-Marquet, G. Wilharm, M.-H. Laaberki and X. Charpentier, *Journal of Bacteriology*, 2018, **200**, e00181-18.

[2] M. Kochar, M. Crosatti, E. M. Harrison, B. Rieck, J. Chan, C. Constantinidou, M. Pallen, H.-Y. Ou and K. Rajakumar, *Antimicrobial agents and chemotherapy*, 2012, **56**, 1845–1853.

P3-23: Isolation of airborne carbapenem resistant *Acinetobacter baumannii* from Intensive care unit of a tertiary regional care hospital in Kuwait

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Back ground

Kuwait, a country in the Middle East is affected regularly by sand storms. Occasionally, sand storms could result in closures of operating theatres due to high levels of dust in the atmosphere. Identifying if hospital air contains any clinically resistant bacteria is important as it might contribute to the transmission of pathogens in clinical settings. Despite the challenges related to surviving in the atmosphere many microbes can remain viable in air even after extended periods of time.

Materials and methods

Air samples (30 L/min) were collected from intensive care unit (ICU) by SKC Biostage™ device sucking air for 15 minutes onto nutrient agar containing 50µg/L cycloheximide and 10µg/L nystatin and 10µg/ml of one of these antibiotics: erythromycin, streptomycin, tetracycline and nalidixic acid. The device was located 1 m above the ground surface. Plates were incubated at 22°C for 3-4 days. Genomic DNA extractions of single isolated colonies from each antibiotic plate were amplified using 16SrRNA primers. Amplified DNA fragments were then purified and sequenced. Identification at the species level was performed by comparison with the Ribosomal Database Project database (<http://rdp.cme.msu.edu/>) and by using BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Antibiotic susceptibility testing was performed by disk diffusion method and MICs were determined with E-test.

Results

Thirty-nine antibiotic resistant bacterial isolates were obtained from ICU. Of which 4 (15.6%) were identified as *A. baumannii*. All four were resistant to imipenem and meropenem (MIC>16). They were resistant to all the antibiotics tested except for colistin.

Conclusions

Carbapenem-resistant *A. baumannii* isolates found in ICU raise serious concerns especially among immune-compromised patients. Although in this hospital infection control practices is adhered to, it is essential to vigilantly monitor the quality of air.

P3-24: Characterization of the novel OXA-213-like carbapenemase OXA-822 from *Acinetobacter calcoaceticus*

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Members of the *Acinetobacter calcoaceticus/baumannii* complex are opportunistic pathogens with the ability to provoke severe infections. Their outstanding extended antibiotic resistance can be either caused by intrinsic resistance determinants such as the *Acinetobacter*-derived cephalosporinases (ADC) or acquisition of new resistance determinants. This study analysed the novel carbapenem-hydrolysing class D beta-lactamase OXA-822 identified in the clinical *A. calcoaceticus* isolate AC_2117.

Whole genome sequencing (PacBio) was performed to elucidate phylogenetic relatedness and for resistome analysis. Antibiotic susceptibility of AC_2117 and transformants harbouring cloned *bla*_{OXA-822} was evaluated by microbroth dilution. OXA-822 was heterologously expressed, purified and enzyme kinetic parameters were determined using spectrophotometry. The *Galleria mellonella in vivo* infection model was used to evaluate the impact of OXA-822 on meropenem therapy.

Analysis of the resistome of AC_2117 revealed the presence of a yet undescribed oxacillinase, termed OXA-822. It is a member of the intrinsic OXA-213-like family from *A. calcoaceticus* and *A. pittii*. The sequence identity on amino acid level to the next related OXA-359 was 96%. Cloning of *bla*_{OXA-822} and expression in *A. baumannii* ATCC 19606 resulted in elevated MICs for carbapenems (up to 8-fold). In contrast, no impact on MICs for carbapenems and cephalosporins could be detected in *A. calcoaceticus* and *E. coli*. Penicillinase activity of the purified OXA-822 revealed high K_m values in the millimolar range paired with high turnover numbers. OXA-822 showed highest affinities to carbapenems. Affinity for imipenem was ~10-fold higher compared to all other carbapenems, but turnover numbers were not affected. Molecular modelling revealed that imipenem does not interact with a negatively charged side chain of OXA-822 like doripenem leading to a lower affinity, but not influencing the hydrolysis of the beta-lactam ring. Presence of OXA-822 deteriorated survival of infected *G. mellonella* larvae after treatment with meropenem. Only $52.7 \pm 7.7\%$ of the larvae survived after 24 h compared to $90.9 \pm 3.7\%$ survival of the respective control group.

We identified *bla*_{OXA-822} in a clinical *A. calcoaceticus* which represents a novel member of the OXA-213-like family displaying unique enzyme kinetic properties. OXA-822 showed carbapenemase activity by elevating MICs in *A. baumannii* and severely interfered with meropenem therapy *in vivo*.

P3-25: Identification and phenotypic characterization of *Acinetobacter bereziniae* isolates harboring a genomically integrated NDM-1 gene

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Acinetobacter bereziniae has been listed as an opportunistic pathogen in healthcare-associated infections (HAIs), though its incidence of infections and molecular determinants of pathogenesis remain under-examined. Existing automated pathogen identification technologies used in medical laboratories generally lack the resolution for detecting *A. bereziniae*, leading to frequent misreports as *A. baumannii* or *A. guillouiae*. By using a combinatorial approach of PCR- and whole genome sequencing (WGS)-based analyses, for the first time, we showed that *A. bereziniae* possesses an unusual arsenal of antimicrobial resistance (AMR) elements including a genome-integrated NDM-1 gene. NDM-1 carriage in clinical isolates of *A. bereziniae* has been sporadically reported in South Asia and South America [1-2]. Based on gene amplification (*rpoB*, 16S) and confirmatory WGS, we found that *A. bereziniae* probably constitutes at least 1% of a total pool of *Acinetobacter* isolates ($n = 550$) reported as *A. baumannii* by automated systems. Strikingly, out of 5 confirmed *A. bereziniae* clinical isolates (2015-2017), 3 showed extensive drug resistance (XDR) for multiple antibiotics including carbapenems, aminoglycosides and quinolones and harbored a genomically integrated NDM-1 gene within an IS*Aba*125 cassette. In addition, all *A. bereziniae* isolates contained a distinctive *bla*OXA-301 gene [3-4], while the XDR isolates carried plasmids with *tet39*, *bla*CARB-2, *sul1*, *mph(E)* and *msr(E)* genes. In antibiotic-free liquid culture, *A. bereziniae* grew with a doubling time of about 30 min. In blood agar culture, *A. bereziniae* isolates gave colonies smaller than that of *A. baumannii*, and individual bacterial cells were indeed visibly smaller in electron microscopy. Weakly responsive to blue light, the isolates could be readily eradicated by UV irradiation, oxidant disinfectants, and ethanol. In brief, *A. bereziniae* appears to be a fast growing bacterium with an ability to acquire AMR via plasmids and transposition and thus should be closely monitored as an emerging pathogen in regions such as China, where the antibiotic pipeline is fast draining.

[1] Jones *et al.* Characterization of plasmids in extensively drug-resistant *Acinetobacter* strains isolated in India and Pakistan. *Antimicrobial Agents and Chemotherapy* 2015, **59** (2), 923.

[2] Brovedan *et al.* Complete sequence of a blaNDM-1-harboring plasmid in an *Acinetobacter bereziniae* clinical strain isolated in Argentina. *Antimicrobial Agents and Chemotherapy* 2015, **59** (10), 6667.

[3] Zander, E.; Seifert, H.; Higgins, P. G. Insertion sequence IS18 mediates overexpression of blaOXA-257 in a carbapenem-resistant *Acinetobacter bereziniae* isolate. *Journal of Antimicrobial Chemotherapy* 2014, **69** (1), 270.

[4] Périchon *et al.* Identification of 50 class D β -lactamases and 65 *Acinetobacter*-derived cephalosporinases in *Acinetobacter* spp. *Antimicrobial Agents and Chemotherapy* 2014, **58** (2), 936.

P3-26: First case of *bla*_{NDM}-positive *Acinetobacter johnsonii* in Latin America: A potential dissemination source?

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Acinetobacter johnsonii is commonly found in the aquatic environment, and occasionally colonize and produce infections in humans [1], such as catheter-associated bloodstream infections [2]. Even though multidrug-resistance is not common in non-*baumannii* *Acinetobacter* species, it has been evidenced that *A. johnsonii* can acquire antibiotic-resistance genes from different bacterial species and genus [2]. On the other hand, NDM-like carbapenemases have not been reported in clinically-relevant Gram negative bacteria in Chile, in which VIM-, KPC- and OXA-carbapenemases are predominant [3]–[5].

Due to the above, the aim of this work was to study the genetic platform harboring the *bla*_{NDM-like} gene in the first NDM-positive *A. johnsonii* isolate in Latin America.

*bla*_{NDM}-positive *A. johnsonii* strain UCO-489 was recovered from surveillance of the clinical environment in a Hospital in Santiago, Chile. The isolate was identified by MALDI-TOF and corroborated by molecular techniques [6], [7]. Antibiotic resistance profiles were determined by the disk diffusion method and the minimum inhibitory concentrations (MICs) to carbapenems were carried out by E-test. Antibiotic-resistance genes were screened by PCR. Plasmids were characterized by the S1-nuclease method and plasmid DNA extracts were used for PCR analyses.

A. johnsonii UCO-489 was resistant to penicillins, cephalosporins and carbapenems, and susceptible to aztreonam, aminoglycosides, quinolones, tetracycline and trimethoprim/sulfamethoxazole. Five plasmids, ranging from ca. 11 kb to 200 kb, were present in UCO-489, whereas *bla*_{NDM-like} was detected in plasmid DNA extracts. In addition, extended-spectrum β -lactamases were not identified.

Our findings represent the first identification of NDM-like carbapenemases in *A. johnsonii* in Latin America. Since *bla*_{NDM-like} was detected in a plasmid, its potential to be disseminated is high. In consequence, *A. johnsonii* could be involved in the dissemination *bla*_{NDM-like} in the hospital environment among different species, thus surveillance protocols should be strengthened in order to control its spread.

[1] Y. Feng, P. Yang, X. Wang, and Z. Zong, "Characterization of *Acinetobacter johnsonii* isolate XBB1 carrying nine plasmids and encoding NDM-1, OXA-58 and PER-1 by genome sequencing," *J. Antimicrob. Chemother.*, vol. 71, no. 1, pp. 71–75, 2016.

[2] S. Montaña *et al.*, "The genetic analysis of an *Acinetobacter johnsonii* clinical strain evidenced the presence of horizontal genetic transfer," *PLoS One*, vol. 11, no. 8, pp. 1–20, 2016.

[3] W. Wu, Y. Feng, G. Tang, F. Qiao, A. McNally, and Z. Zong, "NDM Metallo- β -Lactamases and Their Bacterial Producers in Health Care Settings," *Clin. Microbiol. Rev.*, vol. 32, no. 2, pp. 1–45, 2019.

[4] A. Opazo-Capurro *et al.*, "Evolutionary dynamics of carbapenem-resistant *Acinetobacter baumannii* circulating in Chilean hospitals," *Infect. Genet. Evol.*, vol. 73, no. November 2018, pp. 93–97, 2019.

[5] A. Vera *et al.*, "KPC: *Klebsiella pneumoniae* carbapenemasa, principal carbapenemasa en enterobacterias," *Rev. Chil. infectología*, vol. 34, no. 5, pp. 476–484, 2017.

[6] W. Kamolvit, P. G. Higgins, D. L. Paterson, and H. Seifert, "Multiplex PCR to detect the genes encoding naturally occurring oxacillinases in *Acinetobacter* spp.," *J. Antimicrob. Chemother.*, vol. 69, no. 4, pp. 959–963, 2014.

[7] V. Gundi, L. Dijkshoorn, S. Burignat, D. Raoult, and B. La Scola, "Validation of partial *rpoB* gene sequence analysis for the identification of clinically important and emerging *Acinetobacter* species.," *Microbiology*, vol. 155, no. Pt 7, pp. 2333–41, Jul. 2009.

P3-27: First Detection of Carbapenemase-producing *Acinetobacter baumannii* clinical isolates at Sylvanus Olympio Teaching Hospital in Lome-Togo

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Carbapenems are last resort antibiotics for the treatment of multidrug resistant Gram negative bacteria. However, nowadays, carbapenem resistance have been increasingly reported. In African countries, carbapenemase-producing *Acinetobacter baumannii* were previously reported mainly in North Africa and more rarely in East Africa. Nevertheless, In West Africa, Epidemiological data remains scarce, with only reports of OXA-23-producing *A. baumannii* in Senegal. The aim of this study is to analyse carbapenemases producing *A. baumannii* in Lome, Togo.

During four months study, (April to September 2016), twelve imipenem non-susceptible isolates have been collected. Their Imipenem MIC range from 16 to 128 mg/l. Seven isolates were recovered from hospitalized patients (n=7 ; 58.33%) whereas remaining isolates were recovered from patient at the admission.

All Isolates were fully sequenced using Illumina's technology. Resistome analysis indicated that ten isolates produced carbapenem-hydrolyzing oxacillinase OXA-23 and, among them, seven also produced the metallo-beta-lactamase NDM-1. The two remaining isolates produced OXA-58 and OXA-420, a variant of OXA-58. In addition, two isolates co-produced the ESBL CTX-M-15. All isolates co-producing NDM-1 and OXA-23 belonged to ST1, two isolates belonged to ST103 and the remaining isolates belonged to new STs according to Pasteur's Institute MLST scheme.

We described here, the first description of twelve carbapenemase-producing *A. baumannii* isolated during four months in 2016 at university hospital in Lomé (Togo).

P3-28: Antibiotic resistance and efflux pump inhibitor effect in *Acinetobacter baumannii* strains isolated from Cajamarca, Peru

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Acinetobacter baumannii is a nosocomial pathogen that thrives alarmingly in intensive care units due to its ability to adapt and develop mechanisms of antibiotic resistance [1]. Recent studies in Peru show that most infections are caused by multi-resistant (MDR) and extremely resistant (XDR) strains of this pathogen [2]. In this study, the antibiotic susceptibility of 47 *A. baumannii* strains isolated from Cajamarca, Peru was evaluated.

Antibiotic susceptibility was assessed by disk diffusion on Mueller-Hinton agar plates, following the Clinical and Laboratory Standards Institute (CLSI) guidelines for the following antibiotics: ampicillin-sulbactam, piperacillin-tazobactam, cefotaxime, ceftazidime, cefepime, gentamicin, amikacin, levofloxacin, doxycycline, tetracycline, meropenem, imipenem, trimethoprim-sulfamethoxazole and colistin. All the isolates were resistant to at least one antibiotic agent in three or more categories. One isolate (2.13%) proved to be resistant to all the antibiotics tested and was classified as pan-drug-resistant (PDR). 25 isolates (53.19%) presented an XDR phenotype, the majority being susceptible to colistin. The remaining 21 isolates (44.68%) were considered MDR.

The effect of phenylalanine-arginine B-naphthylamide on the Minimum Inhibitory Concentration (MIC) of levofloxacin, tetracycline and amikacin was determined. Based on a 4-fold or greater reduction as the criterion for significance [3], 8.51% of the isolates (4/47) showed a significant reduction with levofloxacin, 59.57% (28/47) with tetracycline and 31.91% (15/47) with amikacin.

[1] Harding CM, Hennon SW, Feldman MF. Uncovering the mechanisms of *Acinetobacter baumannii* virulence. *Nat Rev Microbiol*. 2018;16(2):91-10

[2] Levy-Blitchtein S, Roca I, Plasencia-Rebata S, Vicente-Taboada W, Velásquez-Pomar J, Muñoz L, et al. Emergence and spread of carbapenem-resistant *Acinetobacter baumannii* international clones II and III in Lima, Peru. *Emerg Microbes Infect*. 2018 Jul 4;7(1):119.

[3] Pannek, S., P. G. Higgins, P. Steinke, D. Jonas, M. Akova, J. A. Bohnert, H. Seifert, and W. V. Kern. 2006. Multidrug efflux inhibition in *Acinetobacter baumannii*: comparison between 1-(1-naphthylmethyl)-piperazine and phe-nyl-arginine-β-naphthylamide. *J. Antimicrob. Chemother*. 57:970–974.

P3-29: Effect of efflux pump inhibitor on the Minimum Inhibitory Concentration in *Acinetobacter baumannii* strains isolated from Lima, Peru

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Acinetobacter baumannii is a nosocomial pathogen capable of developing antibiotic resistance mechanisms, including resistance to broad-spectrum β -lactams and carbapenems [1]. A recent study in Peru found that all *A. baumannii* isolates studied were resistant to at least one from the three different antibiotic classes that were tested [2].

One of the resistance mechanisms presented by *A. baumannii* is the efflux pumps production, which reduce the concentration of antibiotics and toxic compounds within the bacterial cell [3]. It was shown that the use of efflux pump inhibitors such as phenylalanine-arginine B-Naphthylamide (PABN) potentiates the activity of certain antibiotics [4].

In this study, the variation of the Minimum Inhibitory Concentration in presence and absence of PABN was evaluated in 19 strains isolated from a national reference hospital in Lima, Peru. It was observed that 10.52% (2/19) of the strains showed a significant reduction with tobramycin, 26.32% (5/19) with ciprofloxacin, 36.84% (7/19) with levofloxacin and nalidixic acid, and 42.11% (8/19) with amikacin. These results could suppose that the mechanism of efflux pumps is important for the development of multiresistant *A. baumannii* strains.

[1] Maragakis LL, Perl TM, Perl TM. Antimicrobial Resistance: *Acinetobacter baumannii*: Epidemiology, Antimicrobial Resistance, and Treatment Options. *Clin Infect Dis*. 2008 Apr 15 [cited 2018 Aug 8];46(8):1254–63.

[2] Levy-Blitchtein S, Roca I, Plasencia-Rebata S, Vicente-Taboada W, Velásquez-Pomar J, Muñoz L, et al. Emergence and spread of carbapenem-resistant *Acinetobacter baumannii* international clones II and III in Lima, Peru. *Emerg Microbes Infect* [Internet]. 2018 Jul 4 [cited 2018 Aug 11];7(1):119.

[3] Bonomo RA, Szabo D. Mechanisms of multidrug resistance in *Acinetobacter* species and *Pseudomonas aeruginosa*. *Clin Infect Dis*. 2006;43 Suppl 2:S49-56.

[4] Pannek S, Higgins PG, Steinke P, Jonas D, Akova M, Bohnert JA, et al. Multidrug efflux inhibition in *Acinetobacter baumannii*: comparison between 1-(1-naphthylmethyl)-piperazine and phenyl-arginine-beta-naphthylamide. *J Antimicrob Chemother*. 2006;57(5):970-4

P3-30: Generation and selection of antibodies for a immunochromatographic lateral flow assay to rapidly identify OXA-23-, OXA-40- and OXA-58-like mediated carbapenem-resistance in *Acinetobacter baumannii* isolates

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Treatment of *A. baumannii* infections can be extremely challenging owing to the wide distribution of multi-drug resistant strains. Of special concern is increasing resistance against carbapenems, which are now reported worldwide. As a matter of fact, the WHO published a global list of antibiotic-resistant priority pathogens with carbapenem-resistant *A. baumannii* on “priority 1” for research, discovery and development of new antibiotics [1]. Colonization with carbapenem-resistant *A. baumannii* (CRAb) requires rapid action from an infection control perspective because the organism is known for its propensity for epidemic spread. Hence, there is an unmet medical need to rapidly identify CRAb to assist in appropriate antibiotic treatment and to prevent transmission.

CRAb is mainly mediated by the acquisition of the carbapenemase OXA-23. In a “Proof-of-concept” study we have generated and selected anti-OXA-23 monoclonal antibodies (moabs), which have been implemented in an OXA-23 immunochromatographic lateral flow test (ICT). The OXA-23 K-SeT, launched in July 2018, has demonstrated to give reliable results within 15 min with 100% specificity on clinical isolates.

Our aim is to expand the OXA-detection abilities of the OXA-23 K-SeT to OXA-40/OXA-58-like carbapenemases. Therefore, we generated, selected and purified specific anti-OXA-40 and anti-OXA-58 moabs and characterized them in an ICT format. All possible combinations of purified, specific anti-OXA-40 (n=8) and anti-OXA-58 (n=6) moabs, were analyzed in an ICT format for their ability to detect recombinant OXA-40 or OXA-58, respectively. Antibody pairs (capture and detection) showing specific and strong signals were chosen and implemented into single-ICT-prototypes. Those single ICT-prototypes for OXA-40 and OXA-58, were evaluated on clinical *A. baumannii* with well-defined carbapenem resistance mechanisms.

Based on the ICT-prototype validation the development of a triple-OXA-23/40/58 ICT can be envisaged to detect more than 93 % of CRAb strains worldwide. With this rapid detection assay one can save 12-48 hours in diagnostics, which helps to treat earlier with appropriate antibiotics and allows immediate intervention to control transmission of CRAb.

[1] Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. 27. February 2018, online available: http://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf

P4-1: *Acinetobacter baumannii* porin CarO: transcriptional response to growth phase, carbapenems and host cells

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Acinetobacter baumannii has been recognized as one of the most serious pathogens in clinical settings worldwide. The success of this pathogen has been enabled by intrinsic and acquired resistance to antibiotics and virulence [1]. It is considered that porins, outer membrane proteins, could contribute to *A. baumannii* pathogenic potential via antibiotic resistance and/or virulence [2,3]. The role of carbapenem resistance-associated outer membrane protein (CarO) in carbapenem resistance was established previously [4], while the involvement in virulence was indicated [5]. Transcriptional response of the *carO* gene in carbapenem-sensitive and carbapenem-resistant *A. baumannii* strains (813 and 1995/12, respectively) to the different signals was addressed in this study.

The results obtained in this study indicated that transcription of the *carO* gene was growth phase dependent, but the common trend could not be established when comparing results from different strains. However, pattern of the *carO* gene expression in carbapenem-resistant *A. baumannii* 1995/12 was similar to *A. baumannii* ATCC19606 and ATCC17978 [6,7]. Transcriptional response of the *carO* gene to subinhibitory concentration of carbapenems was comparable in both tested strains. Imipenem caused significant decrease in the *carO* gene transcription through different growth phases, that was more pronounced in carbapenem-sensitive strain 813. The effect of meropenem was different since after decreasing in exponential phase increased the *carO* mRNA level in stationary phase. This observation supports the assumption that CarO is porin specific for imipenem, but not meropenem [8]. Potential role of CarO porin in *A. baumannii* virulence was investigated by contact of strains with HaCaT keratinocytes. HaCaT cells significantly induce the *carO* transcription in both strains, especially in carbapenem-sensitive strain 813. The obtained results point out that CarO porin expression is precisely regulated depending on the signals which cell received. Carbapenem presence leads to CarO expression decrease and carbapenem resistance, while HaCaT cells presence stimulates CarO production and potential virulence.

[1] D. Wong, T. B. Nielsen, R. A. Bonomo, P. Pantapalangkoor, B. Luna and B. Spellberg. Clinical and pathophysiological overview of *Acinetobacter* infections: a century of challenges. *Clin. Microbiol. Rev.* **30** (2017), 409-447.

[2] L. Fernández and R. E. Hancock. Adaptive and mutational resistance: role of porins and efflux pumps in drug resistance. *Clin. Microbiol. Rev.* **25** (2012), 661-681.

[3] W. Achouak, T. Heulin and J.M. Pagès. Multiple facets of bacterial porins. *FEMS Microbiol. Lett.* **199** (2001), 1-7.

[4] M. A. Mussi, A. S. Limansky and A. M. Viale. Acquisition of resistance to carbapenems in multidrug-resistant clinical strains of *Acinetobacter baumannii*: natural insertional inactivation of a gene encoding a member of a novel family of β -barrel outer membrane proteins. *Antimicrob. Agents Chemother.* **49** (2005), 1432-1440.

[5] F. Fernández-Cuenca, Y. Smani, M. C. Gómez-Sánchez, F. Docobo-Pérez, F. J. Caballero-Moyano, J. Domínguez-Herrera, A. Pascual and J. Pachón. Attenuated virulence of a slow-growing pandrug-resistant *Acinetobacter baumannii* is associated with decreased expression of genes encoding the porins CarO and OprD-like. *Int. J. Antimicrob. Agents.* **38** (2011), 548-549.

[6] D. Fernando and A. Kumar. Growth phase-dependent expression of RND efflux pump- and outer membrane porin-encoding genes in *Acinetobacter baumannii* ATCC 19606. *J. Antimicrob. Chemother.* **67** (2011), 569-572.

[7] N. C. Soares, M. P. Cabral, C. Gayoso, S. Mallo, P. Rodriguez-Velo, E. Fernández-Moreira and G. Bou. Associating growth-phase-related changes in the proteome of *Acinetobacter baumannii* with increased resistance to oxidative stress. *J. Proteome Res.* **9** (2010), 1951-1964.

[8] M. Catel-Ferreira, G. Coadou, V. Molle, P. Mugnier, P. Nordmann, A. Siroy, T. Jouenne and E. Dé. Structure-function relationships of CarO, the carbapenem resistance-associated outer membrane protein of *Acinetobacter baumannii*. *J. Antimicrob. Chemother.* **66** (2011), 2053-2056.

P4-2: Investigation of the Relation between Colistin Resistance and the Expression of *Acinetobacter baumannii* Virulence factors

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Aim:

Acinetobacter baumannii (AB) has emerged as a problematic multidrug-resistant (MDR) hospital pathogen. Due to the high incidence of carbapenem-resistance, colistin is often the only effective antimicrobial against MDR-AB. However, we are now witnessing the establishment of colistin-resistant (CoR) AB. This study was conducted to investigate the relation between colistin resistance and the fitness and virulence of MDR-AB.

Methods:

Of 139 MDR-AB isolates from clinical specimens in Cairo University Hospitals, 3 isolates were colistin-resistant. We compared the virulence genes of CoR and colistin-sensitive (CoS) isolates by whole genome sequencing (WGS). The virulence and fitness of both CoR and CoS strains were explored under normal and stress conditions (before and after incubation for an hour in pooled normal human serum) through evaluating the expression of some virulence genes including *ompA*, *recA*, *pilD*, *uspA* and *pbp* by real time PCR, and testing for in-vivo fitness in a mouse infection model.

Results:

By WGS, CoR and CoS isolates shared most of the virulence genes (*argG*, *bap*, *fba*, *pgaA*, *pgaB*, *pgaC*, *pilT*, *rpmF*, *ompA*, *recA*, *pilD*, *uspA* and *pbp*), but CoR had an additional 4 virulence genes (*carB*, *sucC*, *surA*, *uvrA*).

The *pbp* and *pilD* genes were over-expressed in CoR strains, both under normal condition (Mean fold change = 3.09 & 3.72, respectively) and after exposure to the stress condition (Mean fold change = 1.13 & 2.97, respectively). The *uspA* gene and *recA* genes were over-expressed in CoR strains only under normal conditions (Mean fold change = 1.35 & 2.23, respectively). The *ompA* gene was under-expressed in CoR strains both under normal condition (Mean fold change = 0.05) and after exposure to the stress condition (Mean fold change = 0.3). There were no statistically significant differences between *ompA*, *recA*, *pilD*, *uspA* and *pbp* gene expression before and after stress ($p = 0.109, 0.109, 1, 0.109, 0.109$, respectively).

In the mouse sepsis model, infection with an inoculum of 1×10^8 CFU resulted in a mortality of 100% with CoR strains, with a mean time to death of 0.5 days, and a mortality rate of 77.8% in mice infected with CoS strains, with a mean time to death of 1.9. There were no statistically significant differences in virulence between CoR and CoS isolates using the log rank test ($P=0.145$).

Conclusion:

We found that CoR isolates possessed more virulence genes. Moreover, some of the virulence associated genes were overexpressed in the CoR isolates.

Key words:

Acinetobacter baumannii, colistin, resistance, virulence genes, fitness.

P4-3: A *rsaM* homolog regulates quorum sensing and virulence in *Acinetobacter baumannii*

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Acinetobacter baumannii is a gram-negative nosocomial pathogen that mostly causes ventilator-associated infections, bloodstream infections and soft tissue infections in seriously ill patients [1,2]. Even though several virulence-associated factors have been described [3], the exact mechanisms of infection and the overall regulation of *A. baumannii* virulence are still poorly understood.

In this project we investigated the role of *ABUW_3775*, a gene encoding an *rsaM* homolog, in the hypervirulent *A. baumannii* strain AB5075. RsaM is known to be involved in virulence gene regulation and quorum sensing repression in the plant pathogens *Pseudomonas fuscovaginae* [4] and *Burkholderia cenocepacia* [5], but its role in *A. baumannii* has not been investigated.

Using an *ABUW_3775* transposon mutant, we detected and quantified quorum sensing molecules by LC-MS/MS, revealing a 100-fold increase in the main signal (3-OH-C12-HSL) when compared with the wild-type parental strain. This transposon mutant also exhibited significantly higher motility in 0.3% Eiken agar and higher attachment to polystyrene. Additionally, *ABUW_3775* was required for full virulence in *Galleria mellonella* larvae. A promoter fusion with the *lux* operon showed that *ABUW_3775* expression is modulated by OH-C12-HSL, highlighting the connection between quorum sensing and *ABUW_3775*.

Overall, our work suggests that *ABUW_3775* plays an important role in the regulation of quorum sensing and virulence of *A. baumannii*. Ongoing RNA-seq studies, as well as future work involving protein-protein interaction assays, will give us more insights about the function of *ABUW_3775* and its role in the biology of *A. baumannii*.

[1] Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*: Emergence of a successful pathogen. *Clin Microbiol Rev* (2008); **21**:538–82.

[2] Antunes LCS, Visca P, Towner KJ. *Acinetobacter baumannii*: Evolution of a global pathogen. *Pathog Dis* (2014); **71**:292–301.

[3] Harding CM, Hennon SW, Feldman MF. Uncovering the mechanisms of *Acinetobacter baumannii* virulence. *Nat Rev Microbiol* (2018); **16**:91–102.

[4] Mattiuzzo M, Bertani I, Ferluga S, Cabrio L, Bigirimana J, Guarnaccia C, et al. The plant pathogen *Pseudomonas fuscovaginae* contains two conserved quorum sensing systems involved in virulence and negatively regulated by RsaL and the novel regulator RsaM. *Environ Microbiol* (2011); **13**:145–62.

[5] Silja Inhülsen aus Hannover von, Promotionskomitee Leo Eberl Kathrin Riedel Jakob Pernthaler D. Investigations of the Quorum Sensing Circuitry in *Burkholderia cenocepacia* H111. PhD dissertation. *University of Zurich*. (2011)

P4-4: The sensor kinase BfmS attributes to the regulation of outer membrane vesicles in *Acinetobacter baumannii*

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Two-component system BfmRS plays a role in virulence and antimicrobial resistance of *Acinetobacter baumannii* through the regulation of bacterial envelope structures [1,2]. This study investigated the role of a sensor kinase BfmS in the outer membrane vesicle (OMV) production and the localization of outer membrane protein A (OmpA) in either outer membrane or OMVs using the wild-type *A. baumannii* ATCC 17978, $\Delta bfmS$ mutant, and *bfmS*-complemented strains.

The $\Delta bfmS$ mutant was constructed by markerless, in-frame deletions [3]. The $\Delta bfmS$ mutant showed hypermucoid phenotype and growth retardation under static culture conditions. The expression of the *bfmR* and *csuCD* genes, biofilm formation, and adherence to host cells were not different between the wild-type and $\Delta bfmS$ mutant strains, but the $\Delta bfmS$ mutant showed the reduced susceptibility to aztreonam and colistin compared to the wild-type stain. The $\Delta bfmS$ mutant produced 4.5 times more OMVs than the wild-type stain. The $\Delta bfmS$ mutant expressed lesser OmpA in the outer membrane, but released more OmpA through OMVs than the wild-type strain, even though the *ompA* gene expression was not different between the two strains. The OMVs of $\Delta bfmS$ mutant were more cytotoxic towards A549 cells than OMVs of the wild-type stain.

Our results suggest that sensor kinase BfmS controls the OMV production and the localization of OmpA in either outer membrane or OMVs, which may contribute to OMV-mediated pathogenesis of *A. baumannii*.

[1] C. Kröger, SC. Kary, K. Schauer, AD. Cameron. Genetic regulation of virulence and antibiotic resistance in *Acinetobacter baumannii*. *Genes*. **8** (2016), E12.

[2] E. Geisinger, NJ Mortman, G. Vargas-Cuebas, AK. Tai, RR. Isberg. A global regulatory system links virulence and antibiotic resistance to envelope homeostasis in *Acinetobacter baumannii*. *PLoS Pathog.* **14** (2018), e1007030.

[3] MH. Oh, JC. Lee, J Kim, CH Choi, K Han. Simple method for markerless gene deletion in multidrug-resistant *Acinetobacter baumannii*. *Appl. Environ. Microbiol.* **81** (2015), 3357-3368.

P4-5: The pathogenic role of the A1S_3412 gene, encoding a D-alanyl-D-alanine carboxypeptidase, in *Acinetobacter baumannii*

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Acinetobacter baumannii is a notorious nosocomial pathogen that commonly infects severely ill patients in intensive care units [1,2]. However, the pathogenesis of *A. baumannii* has not been fully characterized. This study investigated the pathogenic role of the A1S_3412 gene, a zinc uptake regulator-regulated gene [3], using the wild-type *A. baumannii* ATCC 17978, Δ A1S_3412 mutant, and A1S_3412 gene-complemented strains.

The A1S_3412 gene was predicted to encode a D-alanyl-D-alanine carboxypeptidase (DD-CPase) and was highly conserved among *A. baumannii* strains, with 98-100% homology. Recombinant A1S_3412 protein showed specific DD-CPase activity. Expression of the A1S_3412 gene was significantly higher in biofilm cells than in planktonic cells. The Δ A1S_3412 mutant showed reduced biofilm formation, surface motility, and adherence to and invasion of epithelial cells compared to the wild-type strain. In a mouse pneumonia model, the Δ A1S_3412 mutant showed significantly lower bacterial numbers in the blood than the wild-type strain. These virulence traits were restored in the A1S_3412 gene-complemented strain. Under static conditions, the expression of *csuCDE*, which are involved in the chaperone-usher pili assembly system [4], was significantly lower in the Δ A1S_3412 mutant than in the wild-type strain. Moreover, the expression of the *bfmR/S* genes, which regulate the CsuA/BABCDE system, was significantly lower in the Δ A1S_3412 mutant under static conditions than in the wild-type strain.

Our results indicate that the A1S_3412 gene plays a role in *A. baumannii* pathogenesis by regulating the BfmR/S two-component system and subsequently the CsuA/BABCDE chaperone-usher pili assembly system, suggesting it as a potential target for anti-virulence strategies against *A. baumannii*.

[1] L. Dijkshoorn, A. Nemeč and H. Seifert. An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*. *Nat. Rev. Microbiol.* **5** (2007), 939-951.

[2] AY. Peleg, H. Seifert, and DL. Paterson. *Acinetobacter baumannii*: emergence of a successful pathogen. *Clin. Microbiol. Rev.* **21** (2008), 538-582.

[3] MI. Hood, BL. Mortensen, JL. Moore, et al. Identification of an *Acinetobacter baumannii* zinc acquisition system that facilitates resistance to calprotectin-mediated zinc sequestration. *PLoS Pathog.* **8** (2012), e1003068.

[4] AP. Tomaras, CW. Dorsey, RE. Edlmann, LA. Actis. Attachment to and biofilm formation on abiotic surfaces by *Acinetobacter baumannii*: involvement of a novel chaperone-usher pili assembly system. *Microbiology* **149** (2003), 3473-3484.

P5-1: Development of vectors for transcriptional analysis in multidrug-resistant *Acinetobacter* species

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Acinetobacter genus includes several species of opportunistic pathogens. *Acinetobacter baumannii* is renowned for being multidrug-resistant (MDR) and worldwide distributed, especially in hospitals. Despite the clinical relevance of MDR *Acinetobacter* spp., the regulation of their pathogenicity is still poorly understood due to lack of adequate genetic tools, including optimized vectors for gene expression analysis. We report the construction and testing of a series of *Escherichia coli*-*Acinetobacter* spp. promoter-probe vectors suitable for transcriptional analysis in *Acinetobacter* spp. These plasmid vectors, called pLPV1Z, pLPV2Z and pLPV3Z, encode for both gentamicin and zeocin resistance, and contain *lux*, *lacZ* and GFP reporter systems downstream of an extended multilinker. The pLPV plasmids are characterized for being: *i*) 5.5 to 10.3 kb in size; *ii*) very stable in the absence of antibiotic selection due to carriage of a toxin-antitoxin system; *iii*) easily transferable by electroporation into MDR *A. baumannii* strains having different genetic backgrounds (international clonal lineages 1, 2 and 3) as well as in *A. baumannii* complex spp. The pLPV vectors can be extracted with high yields from both *E. coli* and *A. baumannii*, consistent with their high copy number (50-70 copies). Here, pLPV vectors have successfully been used to investigate the iron-regulated promoter of the acinetobactin biosynthesis gene *basA* [1] (A1S_2391) and the DNA damage-inducible promoter of the *uvrA* operon [2] (A1S_3295). Our results indicate that pLPV plasmids are valuable tools for transcriptional studies in *Acinetobacter* spp., and therefore they hold promise for gene expression analysis in this genus.

[1] B.A. Eijkelkamp, K.A. Hassan, I.T. Paulsen and M.H. Brown. Investigation of the human pathogen *Acinetobacter baumannii* under iron limiting conditions. *BMC Genomics* **12** (2011), 126.

[2] J. Aranda, M. Poza, M. Shingu-Vázquez, P. Cortés, J.D. Boyce, B. Adler, J. Barbé and G. Bou. Identification of a DNA-damage-inducible regulon in *Acinetobacter baumannii*. *J Bacteriol.* **195** (2013), 5577-82.

P5-2: The UDP-GalNacA sugar biosynthesis genes *tviBC* are required to maintain the cell envelope integrity and *in vivo* fitness in multi-drug resistant *Acinetobacter baumannii*

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Acinetobacter baumannii infects a wide range of anatomic sites including the respiratory tract and bloodstream. The high prevalence of infection and associated multi-drug resistance leaves few, and in some cases, no antimicrobial treatment options. Despite its clinical importance, relatively little is known about the molecular basis of *A. baumannii* pathogenesis. Using transposon sequencing (Tn-Seq) and the neutropenic murine model of bloodstream infection, we identified the *tviBC* genes as critical for survival *in vivo*. *TviBC* are involved in the biosynthesis of UDP-N-acetyl-D-galactosaminuronic acid (UDP-GalNacA). In addition to its role in capsule production, we hypothesized that synthesis of this sugar is important for the cell envelope homeostasis, *in vivo* fitness and resistance to antibiotics. To test these hypotheses, a double *tviBC* mutant was constructed. As expected, capsule production was inhibited in the mutant construct. Since capsule prevents complement-mediated killing, we were not surprised to observe that the mutant was 5.0-logs more susceptible to human serum. Similarly, in the murine model of bacteremia, the mutant was unable to colonize the bloodstream. In addition, the mutant was more susceptible to vancomycin, unable to grow on MacConkey plates, and had a positive phenotype in the hydrolysis of the chromogenic substrate XP assay. These phenotypes are indicative of an alteration of the cell envelope integrity. An analysis of the Lipid A profiles by mass spectrometry showed a modified profile in the mutant strain. Indeed, the tetra- and hexa-acylated species were reduced, while the hepta-acylated species was increased. Work is in progress to quantify the fatty acid compositions of the Lipid A between the WT and the mutant strain. We also determined that the envelope stress response was induced in the mutant, which is concomitant with the idea of a perturbed cell envelope homeostasis in the *tviBC* mutant. Thus, the mutant was more susceptible to several classes of antibiotics including carbapenem, cephalosporin and tetracycline. By creating single *tviB* and *tviC* mutants, we determined that the phenotypes observed in the Δ *tviBC* mutant were mostly attributed to *tviB*. These results contribute to our understanding of the mechanisms of *A. baumannii* pathogenesis, as well as the biogenesis of its cell envelope and ultimately, will allow us to formulate strategies to manage or prevent *A. baumannii* infections.

P5-3: Thioredoxin is a mediator of cell surface hydrophobicity for *Acinetobacter baumannii*

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Acinetobacter baumannii is a leading cause of nosocomial infections in the United States and worldwide. Several bacterial virulence factors associated with *Acinetobacter* infections have been identified. We have previously reported that the thioredoxin (Trx) system plays an important role in bacterial colonization in the gastrointestinal tract [1] and deletion of *TrxA* gene in a clinical *A. baumannii* isolate Ci79 strain (Δ trxA) led to an attenuation in virulence [2]. Due to their low redox-potential, thioredoxins are efficient disulfide reductants and act as a hydrogen donor for key enzymes involved in various cellular functions. Phenotypic assessment of Δ trxA revealed an increase of cell surface hydrophobicity (CSH) as measured by both Microbial Adhesion to Hydrocarbon and salt aggregation. Bacterial CSH has previously been correlated with both virulence and uptake by host immune cells. Treatment of Δ trxA with reducing agents, including sodium cyanoborohydride (SCBH) and β -mercaptoethonal, markedly reduce CSH by complementing the lack of TrxA expression in the mutant strain. Δ trxA was taken up by J774 macrophages more readily than the WT and this differential uptake could be abrogated though SCBH treatment. Additionally, when partitioned into aqueous and hydrophobic sections, Δ trxA recovered from the hydrophobic partition was phagocytosed more readily than the aqueous section, suggesting that TrxA could modulate CSH to reduce bacterial uptake by host phagocytic cells. The association of TrxA deficiency with increased hydrophobicity and uptake by J774 cells was also observed in a second Gram-negative bacterium, *Francisella novicida*. TrxA is involved in many cellular processes which may also contribute to CSH. We previously demonstrated that reduction of type IV pilus system (T4P) was a major *A. baumannii* Δ trxA phenotype. *Francisella* Fn Δ trxA also had a marked T4P deficiency. Interestingly, the *F. novicida* mutant lacking pilT showed increased hydrophobicity over wild type U112 strain. Collective evidence presented in this study suggests that Gram-negative bacterial thioredoxin modulates CSH for immune evasion through multiple mechanisms including disulfide-bond reduction and T4P assembly.

[1] Ketter et al. *Acinetobacter baumannii* gastrointestinal colonization is facilitated by secretory IgA which is reductively dissociated by bacterial thioredoxin A. *mBio* 2018;9.

[2] Ainsworth et al. Vaccination with a live attenuated *Acinetobacter baumannii* deficient in thioredoxin provides protection against systemic *Acinetobacter* infection. *Vaccine* 2017;35:3387-94.

P5-4: Consequences of blocking peptidoglycan synthesis in *Acinetobacter*

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To characterize the consequences of eliminating peptidoglycan synthesis in *Acinetobacter baylyi*, we generated deletion mutations by natural transformation and visualized the resulting microcolonies of dead cells. We found that loss of genes required for peptidoglycan precursor synthesis or polymerization led to the formation of polymorphic, osmotic pressure-sensitive giant cells with diameters that could exceed ten times normal. Treatment with antibiotics targeting early or late steps of peptidoglycan synthesis also produced giant cells. Genome-scale transposon mutant screening (Tn-seq) identified mutations that blocked or accelerated giant cell formation. The mutations define a genetic pathway for formation of the unusual cells. Although the study focused on *A. baylyi*, we found that a pathogenic relative (*A. baumannii* AB5075) also produced giant cells with genetic dependencies overlapping those of *A. baylyi*.

P5-5: Structural and functional studies of enzymes of Co-enzyme A biosynthesis pathway in *Acinetobacter baumannii*

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The co-enzyme A (CoA) biosynthesis pathway is one of the key biosynthesis pathways in bacteria. CoA is used as an essential cofactor in a large number of enzyme catalyzed metabolic reactions. The knockout of the CoA biosynthetic gene has been shown to impair the processes of development and regulation of cell. The biosynthesis of CoA is a five step cascade starting from pantothenate (vitamin B5) to 4'-phosphopantothenate to 4'-phospho-N-pantothenoylcysteine to 4'-phosphopantetheine to dephospho-CoA to CoA. The aim of this work is the determination of the detailed three-dimensional structures of all five enzymes of CoA biosynthesis pathway in the drug resistant bacteria, *Acinetobacter baumannii* in an attempt to fully characterize and map the molecule for designing ligands which may be future drugs as antimicrobial therapeutics. *Acinetobacter baumannii* is an aerobic Gram negative, non-motile bacteria, commonly found in hospitals. Being a hospital pathogen, *Acinetobacter baumannii* frequently infects patients in intensive care unit and emergency wards. It has become resistant to many classes of antibiotics which have led to an imperative need to discover new drug targets within the bacterium and to consequently determine their three-dimensional structures for obtaining the stereochemical details of the binding sites so that new drugs could be designed against them. It consists of five steps which are catalyzed by distinct enzymes. The steps of CoA synthetic pathway are : Phosphorylation of pantothenate to 4'-pantothenate by pantothenate kinase (PK) ; addition of cysteine by phosphopantothenoylcysteine synthetase (PPCS) to yield (*R*)-4'-phospho-N-pantothenoylcysteine ; decarboxylation to 4'-phosphopantetheine by phosphopantothenoylcysteine decarboxylase (PPCDC) ; transfer of adenylyl group from ATP by phosphopantetheine adenylyltransferase (PPAT) to form dephospho-CoA, and phosphorylation by dephosphocoenzyme A kinase (DPCK) to obtain CoA. There are significant dissimilarities between the bacterial and eukaryotic enzymes within the CoA biosynthetic pathway. PPAT and DPCK are expressed separately in bacteria but form a bifunctional enzyme complex in humans. Conversely, PPCS and PPCDC form a bifunctional enzyme complex in most bacteria but are expressed separately in humans. Only PT is expressed alone in all organisms. In addition to the differences in oligomeric states, sequence similarity between the genes encoding orthologous enzymes is low. Hence, there is immense potential of the bacterial enzymes as targets for antibacterial drugs. The need of the moment is to elucidate and study the three dimensional structures of these proteins, and design ligands that could bind to their active sites effectively. All five enzymes have been cloned, expressed and purified. PPAT has been crystallized and its three dimensional structure has been determined. The crystals of *Ab*PPAT were soaked in the solutions containing TSC. It showed that TSC bound to *Ab*PPAT at the ATP binding site and formed several intermolecular contacts including 12 hydrogen bonds. The results of binding studies and the structure of the complex of *Ab*PPAT with TSC clearly indicated a potential role of TSC as an antibacterial agents.

P6-1: *Acinetobacter baumannii* survives in soil for over a year

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The acute community-acquired human infections with *Acinetobacter baumannii* [1] suggest a source of this pathogen outside hospital settings. Due to limited number of attempts, only two studies reported detection of clinically relevant *A. baumannii* in soils [2,3]. This study investigated the long-term survival of *A. baumannii* in soil, to evaluate the soil as a possible environmental reservoir of *A. baumannii*, prediction of *A. baumannii* behaviour in soil, and their potential consequences.

Fresh and sterilized red soil of pH 8.43 from Istria, Croatia was chosen for the experiment. Three carbapenem-resistant isolates (two environmental and one clinical) were separately suspended in the autoclaved commercial spring water. These suspensions were used to adjust the moisture of the soil to maximum water holding capacity and simultaneously to supplement the soil with 6.7 ± 0.3 log CFU/g of *A. baumannii*. Inoculated soils were left to dry in the dark at 22°C naturally, following drying in the desiccator. In both fresh and sterilized soils, *A. baumannii* isolates slightly multiplied when the water content ranged from 32-16 wt%. A drop of soil moisture from 16-5 wt% was accompanied by a sharp decrease in viable *A. baumannii*. At a soil moisture below 5 wt%, viable *A. baumannii* were maintained during a one-year monitoring in fresh (final abundance 2.4 ± 0.4 log CFU/g), as well in sterilized (final abundance 4.4 ± 0.1 log CFU/g) soil. Scanning electron microscopy confirmed the presence of *A. baumannii* biofilm that developed on the soil particles.

Extensively- and pandrug-resistant *A. baumannii* remain viable in soil for over a year. This suggest the soil as a potential source of clinically relevant isolates that poses a threat to people that come into contact with the soil.

[1] C. Dexter, G.L. Murray, I.T. Paulsen and A.Y. Peleg. Community-acquired *Acinetobacter baumannii*: clinical characteristics, epidemiology and pathogenesis. *Expert. Rev. Anti. Infect. Ther.* **13** (2015), 567-573.

[2] J. Hrenovic, G. Durn, I. Goic-Barisic and A. Kovacic. Occurrence of an environmental *Acinetobacter baumannii* strain similar to a clinical isolate in paleosol from Croatia. *Appl. Environ. Microbiol.* **80** (2014) 2860-2866.

[3] J. Hrenovic, G. Durn, M. Seruga Music, S. Dekic, T. Troskot-Corbic and D. Skoric. Extensively and multi drug-resistant *Acinetobacter baumannii* recovered from technosol at a dump site in Croatia. *Sci. Total Environ.* **607-608** (2017) 1049-1055.

P6-2: Long-term survival of *Acinetobacter baumannii* in water

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Acinetobacter baumannii is a notorious hospital opportunistic pathogen that causes life-threatening infections worldwide [1]. It quickly acquires antibiotic resistance and survives adverse environmental conditions such as starvation [2], desiccation [2,3] and a wide array of temperature and pH regimes [4]. The survival of one environmental (pan drug-resistant) and one clinical isolate (extensively-drug resistant) was monitored for the period of one year in commercially available spring water (SW) and diluted (1:100) nutrient broth (DNB). Isolates were incubated at 22 and 4°C at moderate oxygen saturation of 56%. Initial bacterial abundance determined on Mueller-Hinton agar at 42°C/24h was 6.8 ± 0.1 log CFU/mL.

Both *A. baumannii* isolates performed similar and survived for one year in tested conditions. Survival was slightly better in DNB than in SW, but without statistical significance. After one-year exposure to 22°C the average final abundance was 5.0 ± 0.2 log CFU/mL, while at 4°C the final abundance was 1.2 ± 0.3 log CFU/mL. Small translucent colony variants together with normal opaque colonies were recorded as the response to unfavourable temperature of 4°C. *A. baumannii* can survive in refrigerators as well as in suspensions kept at room temperature for a long time, which is important for its persistence and possible transmission in the hospital environment.

[1] K.J. Towner. *Acinetobacter*: an old friend, but a new enemy. *J. Hosp. Infect.* **73** (2009), 355–363.

[2] Z. Bravo, M. Orruño, C. Parada, V.R. Kaberdin, I. Barcina, I. Arana. The long-term survival of *Acinetobacter baumannii* ATCC 19606T under nutrient-deprived conditions does not require the entry into the viable but non-culturable state. *Arch. Microbiol.* **198** (2016), 399–407.

[3] P. Espinal, S. Martí, J. Vila. Effect of biofilm formation on the survival of *Acinetobacter baumannii* on dry surfaces. *J. Hosp. Infect.* **80** (2012), 56–60.

[4] S. Dekic, J. Hrenovic, T. Ivankovic, E. van Wilpe. Survival of ESKAPE pathogen *Acinetobacter baumannii* in water of different temperatures and pH. *Water Sci. Technol.* **78** (2018), 1370–1376.

P6-3: Intrinsically disorder proteins (IDP) of *Acinetobacter baumannii*

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Acinetobacter baumannii is an emerging opportunistic pathogen responsible for healthcare-associated infections. Recently, infections caused by *A. baumannii* have been steadily increasing and account for as much as 3% of all the hospital-acquired infections. The pathogen has significant intrinsic resistance to antibiotics as well as an extraordinary ability to acquire novel new resistance genes from the environment. This trait has led to a significant decrease in our ability to control or treat *A. baumannii* infections. Furthermore, *Acinetobacter spp.* survives much better on dry surfaces including fomites and medical instruments. Although the desiccation tolerance of various *Acinetobacter spp.* has been studied at the phenotypic level, the molecular mechanisms of desiccation tolerance are currently not understood. For survival under desiccated conditions bacteria require a coordinated series of events during dehydration that are associated with preventing oxidative damage and maintaining the native structure of biological macromolecules.

Intrinsically disordered proteins (IDP) play an important role desiccation tolerance in various organisms including budding yeast, plant seeds, nematode worm, and tardigrade. Although IDPs' functional roles in desiccation tolerance are well established, the mechanistic roles for these proteins vary greatly and are poorly understood. In particular, the role of IDP mediated desiccation tolerance has not been evaluated in bacteria. Since nothing is known about IDPs in *A. baumannii*, we wanted to identify these proteins in the bacterial genomes using various disordered predicting algorithms. Towards this end, we have used four *A. baumannii* strains (ATCC 17978, ATCC19606, B8342, and AB5075) to identify IDPs in the genomes. Surprisingly, we found that *A. baumannii* genomes are significantly enriched with IDPs as compared to bacteria that are not particularly desiccation tolerant. Many of these IDPs are unique in terms of primary amino acid sequences, contain repetitive motifs, and are only encoded by *A. baumannii* genomes. Interestingly, we also found several small IDPs that are less than 100 residues. We are currently evaluating the biological significance of these IDPs in desiccation tolerance.

P6-4: Investigation of a non-canonical function of acinetobactin, the major siderophore for *Acinetobacter baumannii*

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The iron acquisition of *Acinetobacter baumannii* by utilizing siderophores at the infection sites is considered as one of the important virulence factors [1]. Currently, three siderophores, acinetobactin, fimsbactin, and baumannoferrin, have been identified from *A. baumannii*, among which acinetobactin has been demonstrated to be the most prevalent in the clinical isolates [2]. In this regard, our laboratory as well as other research groups have been actively pursuing the elucidation of various facets of chemistry and biology of acinetobactin [3,4].

Recently, we have noticed that acinetobactin is capable of binding not only iron, but also other metals including zinc and copper. The binding of a siderophore with non-iron metals is not unprecedented, but its physiological relevance varies among siderophores [5]. In this regard, we began investigating the biological role of a Zn-acinetobactin complex, particularly focusing on probing the potential “zincophore” activity of acinetobactin, and our recent progresses on this matter will be discussed in this presentation.

[1] C. M. Harding, S. W. Hennon and M. F. Feldman. Uncovering the mechanisms of *Acinetobacter baumannii* virulence. *Nat. Rev. Microbiol.* **16** (2018), 91–102.

[2] L. C. S. Antunes, F. Imperi, K. J. Towner and P. Visca. Genome-assisted identification of putative iron-utilization genes in *Acinetobacter baumannii* and their distribution among a genotypically diverse collection of clinical isolates. *Res. Microbiol.* **162** (2011), 279–284.

[3] W. Y. Song, M. H. Oh and H. J. Kim *et al.* Key structural elements for cellular uptake of acinetobactin, a major siderophore of *Acinetobacter baumannii*. *Org. Lett.* **19** (2017), 500–503.

[4] H. Lee, M. H. Oh and H. J. Kim *et al.* Synthesis and characterization of anguibactin to reveal its competence to function as a thermally stable surrogate siderophore for a Gram-negative pathogen, *Acinetobacter baumannii*. *Org. Lett.* **20** (2018), 6476–6479.

[5] T. C. Johnstone, E. M. Nolan. Beyond iron: non-classical biological functions of bacterial siderophores. *Dalton Trans.* **44** (2015) 6320–6339.

P6-5: Biofilm formation and motility affected by the multidrug efflux pump AdeFGH in *Acinetobacter baumannii*

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Acinetobacter baumannii is known for causing hospital-acquired infections, especially in intensive care units [1]. These infections have become increasingly difficult to treat due to the high frequency of multidrug resistant strains [1]. This multidrug resistance is attributed in part to the presence of resistance-nodulation-division (RND) efflux pumps in *A. baumannii* [2]. The presence of AdeFGH, one of the RND efflux pumps, contributes to reduced susceptibility to a variety of antibiotics such as chloramphenicol, fluoroquinolones, trimethoprim, tetracycline and clindamycin [3].

AdeFGH contributes to other important factors that affect the outcome of *A. baumannii* infections. Previously, AdeFGH has been correlated to changes in biofilm formation and a potential link to quorum sensing has been hypothesized [4]. We have shown that increases in biofilm formation are seen in strains overexpressing the *adeFGH* operon which correlates to changes in expression of a two-component system *bfmRS*. BfmR has been shown to regulate the *csu* operon which is important for attachment and biofilm formation in *A. baumannii* [5] and changes in expression of the genes within the operon are observed in accordance to changes to *bfmR* expression.

Motility is also affected by the expression of *adeFGH* and is correlated to changes in expression of the *pil* genes for the type 4 secretion system used for twitching motility in *A. baumannii* [6]. Changes in expression of genes important for virulence of *A. baumannii* such as the phenylacetic acid metabolism pathway have also been observed.

[1] D. Wong, T. B. Nielsen, R. A. Bonomo, P. Pantapalangkoor, B. Luna, and B. Spellberg, "Clinical and pathophysiological overview of *Acinetobacter* infections: A century of challenges," *Clin. Microbiol. Rev.*, vol. 30, no. 1, pp. 409–447, 2017.

[2] C. V. Leus, I. V. Weeks, J. W. Bonifay, V. Smith, L. Richardson, and S. I. Zgurskaya, "Substrate specificities and efflux efficiencies of RND efflux pumps of *Acinetobacter baumannii*," *J. Bacteriol.*, vol. 200, pp. 49–67, 2018.

[3] S. Coyne, N. Rosenfeld, T. Lambert, P. Courvalin, and B. Perichon, "Overexpression of Resistance-Nodulation-Cell Division Pump AdeFGH Confers Multidrug Resistance in *Acinetobacter baumannii*," *Antimicrob. Agents Chemother.*, vol. 54, no. 10, pp. 4389–4393, Oct. 2010.

[4] X. He *et al.*, "Biofilm Formation Caused by Clinical *Acinetobacter baumannii* Isolates Is Associated with Overexpression of the AdeFGH Efflux Pump," *Antimicrob. Agents Chemother.*, vol. 59, no. 8, pp. 4817–4825, Aug. 2015.

[5] A. P. Tomaras, C. W. Dorsey, R. E. Edelman, L. A. Actis, and C. A. Luis Actis, "Attachment to and biofilm formation on abiotic surfaces by *Acinetobacter baumannii*: involvement of a novel chaperone-usher pili assembly system," *Microbiology*, vol. 149, pp. 3473–3484, 2003.

[6] K. M. Clemmer, R. A. Bonomo, and P. N. Rather, "Genetic analysis of surface motility in *Acinetobacter baumannii*," *Microbiology*, vol. 157, pp. 2534–2544, 2011.

P6-6: Role of K⁺ in the physiology of *Acinetobacter baumannii*

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The outstanding trait of *Acinetobacter baumannii* to withstand long periods in dry environments is based, at least partial, on its ability to maintain cell turgor by accumulation of compatible solutes [1]. These solutes are usually used for long time adaptation in bacteria, whereas a fast answer to low water activities is usually the accumulation of K⁺ in the cytoplasm [2,3].

We examined the physiological role of K⁺ in osmotic stress response in *A. baumannii*. This bacterium possesses three major K⁺ transport systems: the low affinity and constitutively produced transporters Trk and Kup as well as the primary active transporter Kdp with its associated and activity regulating two component system KdpDE. Wild type cells grown under K⁺-limitation, did no longer accumulate the compatible solutes glutamate and mannitol, indicating a role of K⁺ in the biosynthesis of these solutes. To study this effect even further we generated K⁺ transporter deletion mutants and analyzed their phenotype. A $\Delta kup\Delta trk$ deletion mutant had a strong growth defect in comparison to the wild type when grown in medium containing 300 mM NaCl. The growth of a Δkdp deletion mutant in K⁺-limited conditions was even abolished.

Intracellular levels of K⁺ were measured *via* atom absorption spectroscopy measurements (AAS). This experiment revealed a strong K⁺ influx after the addition of NaCl to the cell suspension, which confirms K⁺ as the first answer to osmotic stress. Further studies revealed that the intracellular K⁺ concentration is dependent on the extracellular concentrations as well as on the amount of added osmolytes.

[1] Zeidler, S. and Müller, V. (2018) The role of compatible solutes in desiccation resistance of *Acinetobacter baumannii*. e740. <https://doi.org/10.1002/mbo3.740>.

[2] Rößler, M. and Müller, V. (2001) Osmoadaptation in bacteria and archaea: Common principles and differences. *Environ Microbiol.* 3:743-754.

[3] Kempf, B. and Bremer, E. (1998) Uptake and synthesis of compatible solutes as microbial stress responses to high-osmolality environments. *Arch Microbiol.* 170:319-330.

P6-7: Role of phospholipase D3 in adaptation of *Acinetobacter baumannii* to the human host

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The human pathogen *Acinetobacter baumannii* is the cause of hospital acquired infections worldwide. Its resistance to multiple antibiotics and its ability to survive on dry surfaces supports its adaptation to the hospital environment [1]. The first barrier *A. baumannii* encounters during infection are the membranes of host cells. Therefore phospholipids are good candidates for metabolic adaptation to the human host. Degradation and modulation of phospholipids is mediated by phospholipases which have been identified as virulence factors in many organisms [2]. *A. baumannii* encodes three phospholipases D (PLD 1,2,3) which act in a concerted manner during infection of *Galleria mellonella* larvae and support invasion of human lung epithelial cells [3]. Recently we detected a unique lipid composition of *A. baumannii* with relatively high levels of a large variety of monolysocardiolipins (MLCL) and cardiolipins (CL) [4]. A *pld23* double and a *pld123* triple mutant were lacking CL and MLCL whereas a *pld1* single mutant did not produce MLCL. The membrane lipid pattern of the single deletion mutants suggests that the PLD1 plays a role in monolysocardiolipin formation, while PLD2 and PLD3 play a role in both cardiolipin and monolysocardiolipin production. Members of the PLD family share a conserved catalytic H_xK_{xxxx}D_{x6}GS_xN motif, which is present in two copies and forms the catalytic core of the enzyme. The PLD3 of *A. baumannii* only possesses one such conserved catalytic motif and one in which the conserved aspartate is missing. Mutant studies revealed, that both motifs are essential for PLD3 function indicating that, in contrast to the conserved histidine, the conserved aspartate is not functionally essential.

[1] L. Dijkshoorn, A. Nemeč and H. Seifert. An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*. *Nat. Rev. Microbiol.* **5** (2007), 939-951.

[2] D. H. Schmiel and V. L. Miller. Bacterial phospholipases and pathogenesis. *Microbes. Infect.* **1** (1999), 1103-1112.

[3] J. Stahl, H. Bergmann, S. Göttig, I. Ebersberger, B. Averhoff. *Acinetobacter baumannii* virulence is mediated by the concerted action of three phospholipases D. *PLoS One* **10** (2015), e0138360.

[4] P. Lopalco, J. Stahl, C. Annese, B. Averhoff, A. Corcelli. Identification of unique cardiolipin and monolysocardiolipin species in *Acinetobacter baumannii*. *Sci. Rep.* **7** (2017), 2972.

P6-8: Trehalose biosynthesis in *Acinetobacter baumannii*

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Acinetobacter baumannii is well known for its extraordinary resistance to drought and low water activities. To prevent the loss of cellular water under high osmotic pressure *A. baumannii* accumulates the compatible solutes glutamate and mannitol. Trehalose is also produced but to a much smaller extent. Furthermore, trehalose is also accumulated upon high temperatures [1].

Trehalose is synthesized by *A. baumannii* via the OtsA-OtsB pathway. In this pathway trehalose is synthesized in two steps. The trehalose-6-phosphate synthase encoded by *otsA* catalyzes the condensation of glucose-6-phosphate and UDP-glucose, resulting in the formation of trehalose-6-phosphate. In the next step the trehalose-6-phosphate phosphatase encoded by *otsB* cleaves off the phosphate and releases trehalose [1]. Transcriptional analyses reveal that during growth in minimal medium and in presence of 300 mM NaCl the genes coding for *otsB* and *otsA* as well as the genes responsible for mannitol biosynthesis (*mtlD*) are significantly upregulated.

Not only gene expression is regulated by the salt concentration of the medium, also the activity of the enzymes is regulated. While the activity of MtlD is strictly salt dependent [3], OtsA is also active in absence of salt. However, the activity is stimulated by glutamate. This is plausible since glutamate is seen as second messenger in the distribution of the osmotic stress signal [4].

Deletion mutants of *A. baumannii* *otsB* [1], *otsA* and *otsBA* were generated as well as strains overproducing OtsB, OtsA or OtsBA. The phenotype of the mutants and overproduction strains will be reported.

[1] S. Zeidler, J. Hubloher, K. Schabacker, P. Lamosa and V. Müller. Trehalose, a temperature- and salt-induced solute with implications in pathobiology of *Acinetobacter baumannii*. *Environ. Microbiol.* **19** (2017), 5088-5099.

[2] S. Zeidler and V. Müller. Coping with low water activities and osmotic stress in *Acinetobacter baumannii*: significance, current status and perspectives. *Environ. Microbiol.* (2019), doi:10.1111/1462-2920.14565

[3] S. Zeidler, J. Hubloher, P. König, ND. Ngu, A. Scholz, B. Averhoff and V. Müller. Salt induction and activation of MtlD, the key enzyme in the synthesis of the compatible solute mannitol in *Acinetobacter baumannii* (2018), *Microbiologyopen* **7**: e00614.

[4] SH. Saum and V. Müller. Regulation of osmoadaptation in the moderate halophile *Halobacillus halophilus*: chloride, glutamate and switching osmolyte strategies. (2008), *Saline systems* **4**: 4.

P6-9: RNA-Seq reveals co-regulatory actions of DdrR and UmuDAb in *Acinetobacter baumannii*

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Acinetobacter baumannii strains can undergo mutation to antibiotic resistance after DNA damage by only partially understood mechanisms. In most bacteria, DNA damage-inducible genes are under LexA repression until DNA damage triggers LexA self-cleavage, lifting the repression and allowing induction of transcription. However, *Acinetobacter* species have no LexA repressor, although UmuDAb, the UmuD polymerase manager homolog, represses some DNA damage-inducible genes.

RT-qPCR experiments have shown that *ddrR*, a gene that is transcribed divergently from *umuDAb* and unique to *Acinetobacter* species, co-represses UmuDAb-repressed genes. To test whether *ddrR* regulates additional DNA damage-inducible genes, we performed RNA-Seq analysis on *A. baumannii* ATCC 17978 *ddrR::lacZ-KanR* cells. *ddrR* regulated approximately 25 % (n = 39) of the MMC-induced regulon of wild type (WT) 17978 cells. UmuDAb co-regulated 17 of these 39 *ddrR*-regulated genes, which displayed two different patterns of expression in *ddrR* and *umuDAb* mutant strains. One pattern was exemplified by the *umuDC* polymerases and polymerase managers, *umuDAb*, and *ddrR*, all of which are UmuDAb-repressed genes. These genes were de-repressed in the *ddrR* mutant in the absence of DNA damage, suggesting that their co-repression required DdrR as well as UmuDAb. Another group of nine DNA damage-inducible genes was regulated, but not repressed, by both DdrR and UmuDAb. These genes' expression either did not increase, or did not increase to the same induced level as in WT cells, after DNA damage in the *ddrR* mutant. This RNA-Seq analysis also identified 22 DNA damage-inducible genes that were regulated by DdrR but not UmuDAb. They were also dependent upon RecA for their induction, like 99% of the DNA damage-inducible genes in 17978. These genes were located in three chromosomal cryptic prophages and encoded mostly hypothetical phage proteins.

Finally, 57 induced genes were differentially expressed and induced in the *ddrR* mutant but had not been induced in WT cells. This regulon contained multiple genes for DNA replication, recombination, and repair, six transcriptional regulators, five RND efflux, and six transport genes. Many of these were in gene clusters, supporting the existence of an additional regulatory role for DdrR.

These findings suggest multiple possible roles for DdrR, including as a co-repressor of the LexA-like repressor, UmuDAb, in controlling the expression of bacterial error-prone polymerases.

P6-10: Structural basis for *Acinetobacter baumannii* biofilm formation

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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. Biofilm formation is mediated by Csu pili, assembled via the ‘archaic’ chaperone-usheer pathway. The X-ray structure of the CsuC-CsuE chaperone-adhesin pre-assembly complex reveals the basis for bacterial attachment to abiotic surfaces. CsuE exposes three hydrophobic finger-like loops at the tip of the pilus. Decreasing the hydrophobicity of these abolishes bacterial attachment, suggesting that archaic pili use tip-fingers to detect and bind to hydrophobic cavities in substrates. Anti-tip antibody completely blocks biofilm formation, presenting a means to prevent the spread of the pathogen. The use of hydrophilic materials instead of hydrophobic plastics in medical devices may represent another simple and cheap solution to reduce pathogen spread. Phylogenetic analysis suggests that the tip-fingers binding mechanism is shared by all archaic pili carrying two-domain adhesins. The use of flexible fingers instead of classical receptor-binding cavities is presumably more advantageous for attachment to structurally variable substrates, such as abiotic surfaces. Cryo-electron microscopy analysis of the Csu pilus reveals a very unusual zigzag-like structure, which in addition to donor strand complementation is stabilized by a twin-hairpin acceptor pocket contact. This arrangement enables formation of rigid, but very thin fibers, which ideally suited for both the bacterial attachment and 3D biofilm formation.

Reference: Pakharukova N, Tuittila M, Paavilainen S, Malmi H, Parilova O, Teneberg S, Knight S & Zavalov AV (2018) Structural basis for *Acinetobacter baumannii* biofilm formation. ***Proc Natl Acad Sci U S A*** 115, 5558-5563.

P6-11: Transfer of important clinical resistance genes from *Salmonella enterica* to *Acinetobacter* spp. by natural transformation

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Resistance of *Acinetobacter* spp. to last-resort drugs carbapenems and colistin is increasing. Resistance to colistin is usually mediated by chromosomal genes, but a colistin mobile resistance determinant, *mcr-4.3*, was recently identified in *A. baumannii*. Conjugation is not very common among *Acinetobacter* spp. Since some species are naturally competent, the main objective of this study was to unveil the role of natural transformation in colistin-resistance determinants widespread between genetic unrelated species. Colistin minimal inhibitory concentration (MIC) was determined by broth microdilution in clinical *Acinetobacter* spp. isolates and in *A. baylyi* BD413, all naturally competent. According to MIC values, 5 *A. baumannii* and *A. baylyi* were selected as recipient cells in natural transformation assays performed in semisolid medium. DNA from 2 *Salmonella enterica* resistant to colistin isolated from retail meat carrying plasmidic *mcr-1* and *bla_{ctx-M-1}* were used as donor DNA. Transformants were selected in LB media with colistin at 10 and 24 µg/mL or cefotaxime at 30 µg/mL. Change of susceptibility profile was evaluated by broth microdilution or disk diffusion methods. Acquisition of resistance genes was confirmed by PCR.

Colistin MIC from *Acinetobacter* spp. isolates ranged from 3.9 to 250 mg/L. Isolates with lower MIC were used in natural transformation. Dilution of the transformation mix in NaCl 0.85% allowed growth of recipient cells in control media with colistin at 24 µg/mL, though this concentration was higher than the respective MIC; the use of PBS 1x (with 0.08% NaCl) eliminated this colistin tolerance. Transformants were observed in assays with *A. baumannii* A118, 319 and 113 and with *A. baylyi* recipients under colistin selection. Despite the increased colistin MIC, transformants did not acquire the *mcr-1* gene. One transformant of *A. baumannii* A118, selected with cefotaxime, showed reduced susceptibility to cefotaxime and ceftazidime as compared to the recipient and acquired the *bla_{ctx-M-1}* gene; *mcr-1* was not co-acquired.

This study shows that colistin tolerance can be acquired by exposure to NaCl and condition assays must be monitored. Overall, the results highlight that spread of important clinical resistance genes, such as ESBL genes, can occur by natural transformation between genetic divergent species and changes in the colistin susceptibility profile can also occur after uptake of naked DNA of colistin-resistant *S. enterica* by *Acinetobacter* spp.

P6-12: A metal-responsive transcriptional regulator protects *Acinetobacter baumannii* from oxidative stress

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Acinetobacter baumannii is an important opportunistic pathogen that commonly infects critically ill patients in hospital settings. Because of its rapid acquisition of antibiotic resistance, infections caused by *A. baumannii* have become extremely difficult to treat, underlying the importance of identifying new antimicrobial targets for this pathogen. Neutrophils inhibit growth of *A. baumannii* through a variety of mechanisms, including the production of ROS and the release of calprotectin, an immune protein that chelates zinc (Zn), iron (Fe), and manganese (Mn). We previously identified an NRAMP-family Mn transporter, MumT, which is essential for growth of *A. baumannii* during Mn starvation. MumT is encoded within an operon adjacent to a LysR-family transcriptional regulator, MumR. Transcription of *mumT* is dependent on MumR, and is heightened in the presence of calprotectin. Because Mn import is a common defense against oxidative stress, we hypothesized that MumT may play an important role in defense against H₂O₂. Surprisingly, Δ *mumR*, but not Δ *mumT*, was defective for growth in the presence of H₂O₂, suggesting that MumR regulates other genes that promote resistance to H₂O₂ stress. To determine whether MumR acts through the canonical redox-sensitive regulator OxyR, a Δ *mumR* Δ *oxyR* mutant was constructed. This mutant displayed heightened sensitivity to H₂O₂ relative to Δ *mumR* or Δ *oxyR*, indicating that both proteins activate distinct regulons to promote H₂O₂ resistance. RNA-sequencing was performed to define the regulon of MumR, which revealed a role for MumR in regulating several catabolic pathways. Finally, Δ *mumR* exhibited reduced fitness in a murine model of pneumonia that was restored in the absence of neutrophils, suggesting that MumR-regulated gene products are critical for protecting against neutrophil killing. In summary, these results suggest that MumR facilitates resistance to neutrophil killing by activating a transcriptional program that is critical for surviving Mn starvation and oxidative stress. Future directions will be aimed at further characterizing the functions of genes in the MumR regulon under these conditions.

P6-14: Phospho-secretome characterization of *Acinetobacter baumannii* in biofilm

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Acinetobacter baumannii is one of the most problematic opportunist pathogen responsible for many infections worldwide [1]. It presents high adhesion abilities on any types of abiotic and biotic surfaces leading to biofilm development, a mode of growth conferring an additional protection against various treatments and allowing the infection relapse [2].

Characterization of post-translational modifications, such as phosphorylation, may be an interesting way to identify new therapeutic targets, like in a cancer [3], to eradicate this bacterium. Indeed, different examples have demonstrated the implication of phosphorylation in antibiotic resistance, pathogenesis, virulence and persistence in bacteria [4,5].

Here, Ser/Thr/Tyr phospho-secretomes of *A. baumannii* reference strain ATCC17978 in planktonic and biofilm modes of growth were characterized by using a proteomic approach (titanium dioxide enrichment and high-resolution mass spectrometry). In biofilm, we identified a higher number of phosphoproteins (98 proteins, 137 phosphosites) than in planktonic (35 proteins, 52 phosphosites). Phosphorylated proteins identified in biofilm are involved in different biological processes like ion transport, adaptation, bacterial secretion and iron acquisition. These phospho-secretomes will be compared to those of the clinical *A. baumannii* strain AB0057 to potentially highlight specific proteins or regulation pathways for this virulent isolate.

[1] Antunes *et al.* *Acinetobacter baumannii*: evolution of a global pathogen. *Pathog. Dis.* 71(2014), 292-301.

[2] Espinal *et al.* Effect of biofilm formation on the survival of *Acinetobacter baumannii* on dry surfaces. *J. Hosp. Infect.* 80(2012), 56–60.

[3] Richters. Targeting protein arginine methyltransferase 5 in disease. *Future Med. Chem.* 9(2017), 2081-2098.

[4] Yang *et al.* Small-Molecule Inhibitors Targeting Protein SUMOylation as Novel Anticancer Compounds. *Mol. Pharmacol.* 94 (2018), 885-894.

[5] Soares *et al.* Ser/Thr/Tyr phosphoproteome characterization of *Acinetobacter baumannii*: comparison between a reference strain and a highly invasive multidrug-resistant clinical isolate. *J. Proteomics.* 102(2014), 113-24.

P6-15: *Acinetobacter baumannii* – Who wants to be like the others?

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In general, DNA methylation is a useful tool for bacteria to distinguish their own from foreign DNA. In *A. baumannii* we could find a novel DNA-methyltransferase called *Acinetobacter* DNA adenine methyltransferase **A** (AamA) [1]. During a mutant library screening looking for motility deficiencies, a mutant inactivated in the *aamA* gene was identified. After construction of a GST fusion-protein and expression in *E. coli* the AamA was purified and characterized. Via small-angle X-ray scattering it could be shown, that AamA is a 49 kDa monomer. As AamA lacks a corresponding restriction endonuclease, together with the well-known *E. coli* Dam it belongs to the so called orphan methyltransferases [2]. In comparison to *E. coli* Dam, AamA exhibits a lower enzymatic activity as tested in a restriction protection assay. Using single-molecule real-time PacBio Sequencing it could be shown that AamA performs differential methylation depending on the environmental conditions. PacBio sequencing was done for the natural wildtype strain 29D2 and the corresponding *aamA*-mutant under planktonic and surface-associated biofilm conditions.

For the wildtype strain 29D2, specific methylation motifs could be observed in a condition-independent manner and differential methylation and were only methylated in rates of 17-26%. In the *aamA*-mutant none of these specific motifs was found to be methylated. Furthermore there were two “hotspot” regions showing AamA-specific DNA methylation. One of these regions lies upstream the *purK* gene which matches perfectly with our motility deficiency mutant library [1]. On the basis of the differential methylation of AamA we hypothesized the presence of interaction partners which guide the AamA to the place of action. To search for putative interaction partners of AamA, the fusion-protein (GST-AamA) and the GST alone were purified and were given to the bacterial lysates of the wildtype 29D2 and the *aamA*-mutant. Afterwards the lysates were subjected to Glutathione Sepharose® 4 Fast Flow affinity purification. The pull-down samples were loaded on an SDS-gel that was silver-stained. Specific bands which could only be observed in samples where the GST-AamA and bacterial lysates were incubated together in comparison to the controls (GST-AamA without lysate and GST with lysate) were cut out and subjected to mass spectrometry. Results showed a hit for an 18 kDa putative cytoplasmic protein and an Aconitate hydratase 2 with 95 kDa. In *E. coli*, the latter one is known as a moonlighting protein, which can protect its mRNA against degradation under iron scarcity [3]. To characterize this protein, His-tagged Aconitate hydratase was created and purified for small-angle X-ray scattering (SAXS) analysis. However, it aggregated and no clear hint of an interaction with AamA was given. So for further studies it is being considered to make interaction studies applying native page, EMSA or co-immunoprecipitation.

[1] Blaschke U. et al. (2018). *Protein Expression and Purification*. **151**,78-85.

[2] Adhikari A. et al. (2016), *FEMS Microbiol. Rev.* **40**, 575-591

[3] Benjamin JA. and Massé E. (2014), *Nucleic Acids Res.* **42**, 10023-36.

P6-16: Heterogeneity in *Acinetobacter baumannii*: modern clinical isolates versus reference strains

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The opportunistic pathogen *Acinetobacter baumannii* represents a severe public health threat due to its impressive resistance arsenal to the last resort antibiotics and to harsh conditions such as desiccation, disinfectants and the human immune system [1]. Despite an important and well-established clinical relevance [2], the pathogenicity of this biosafety level 2 bacterium remains to be determined [3].

In order to study the virulence and the stress resistance potential, few reference strains are commonly used worldwide, such as the ATCC 17978, ATCC 19606, AB5075 and DSM30011 [3,4,5]. Yet *A. baumannii* bacteria are known to be highly heterogeneous among different isolates at both genetic and phenotypic levels [1,4]. The aims of this project is to compare the reference strains with successful modern clinical isolates and to better understand the molecular mechanisms governing the extreme resistance capabilities of *A. baumannii*.

Phenotypic characterization using a new density gradient-based method strongly suggests that capsule production levels are heterogeneous among modern clinical isolates, which is less the case for the reference strains. Infections using the *Galleria mellonella* multicellular model show that the virulence potential of the modern clinical isolates is also very heterogeneous, ranging from an avirulent background to highly virulent isolates. However, using the biphasic amoeba *Acanthamoeba castellanii* as a versatile phagocytic cellular model, we show that the majority of the *A. baumannii* strains resists phagocytosis by producing a mucoid phenotype.

The project shows that to study specific aspects of *A. baumannii* virulence and resistance arsenal, the reference strains are not representative of the diversity found among the current clinical isolates. In addition, the amoeba *A. castellanii* represents a promising easy-to-use cellular model to overcome the lack of high-throughput infection models for *A. baumannii*. Comparative genomics approaches combined with phenotypic clustering are currently undertaken to decipher the molecular mechanisms governing the resistances and the virulence arsenal of *A. baumannii*.

[1] Wong, D. *et al.*, Clin Microbiol Rev 30, 409-447, (2017).

[2] WHO, Global priority list of antibiotic-resistant bacteria, (2017).

[3] Lee, C. R. *et al.*, Front Cell Infect Microbiol 7, 55, (2017).

[4] Harding, C. M., Hennon, S. W. & Feldman, M. F. Nat Rev Microbiol 16, 91-102, (2018).

[5] Repizo, G. D. *et al.*, Genome Biol Evol 9, 2292-2307, (2017).

P7-1: Molecular epidemiology of Colistin Resistance in *Acinetobacter baumannii* isolates

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A hundred and twenty-one isolates were isolated from clinical and environmental samples in Baghdad-Iraq. Isolates were diagnosed as *Acinetobacter baumannii* by using morphological tests, Vitek-2 system, 16SrRNA PCR amplification and sequencing. All isolates were subjected to molecular testing to detect genes responsible for colistin resistance in *A. baumannii*. Twenty-six isolates out of 121 samples (21.5%) gave positive results with red colony on CHROM agar *Acinetobacter* base for MDR. CHROMagar™ COL-APSE (Paris, France) medium detected 92 (76%) *A. baumannii* isolates colistin resistance with white colony. PCR assay detected *mcr-1*, *mcr-2* and *mcr-3* in 89 (73.5%), 78 (64.5%) and 82 (67.8%) in the *Acinetobacter* isolates respectively. Because of a rapid increase in colistin resistance in *A. baumannii*, there is need to avoid unnecessary clinical use of colistin where possible.

[1] ABDUL MOMIN, M. H. F., BEAN, D. C., HENDRIKSEN, R. S., HAENNI, M., PHEE, L. M. and WAREHAM, D. W. (2017). CHROMagar COL-APSE: a selective bacterial culture medium for the isolation and differentiation of colistin-resistant Gram-negative pathogens. *J. Med. Microbiol.*, 66(11), pp.1554-1561.

[2] ADAMS, M.D., NICKEL, G.C., BAJAKSOUZIAN, S., LAVENDER, H., MURTHY, A.R., JACOBS, M.R. and BONOMO, R.A. (2009). Resistance to colistin in *Acinetobacter baumannii* associated with mutations in the *PmrAB* two-component system. *Antimicrob. Agents Chemother.*, 53(9), pp.3628–3634.

[3] AHMED, O. B.; ASGHAR, A.H. and ELHASSAN, M.M. (2014). Comparison of three DNA extraction methods for polymerase chain reaction (PCR) analysis of bacterial genomic DNA. *African J. Microbiol. Res.*, 8(6), pp.598-602.

[4] AL-KADMY, I. M. S., ALI, A. N. M., SALMAN, I. M. A. and KHAZAAL, S. S. (2018). Molecular characterization of *Acinetobacter baumannii* isolated from Iraqi hospital environment. *New Microbes New Infect.*, 21(C), pp.51-57.

[5] BALAJI, V., JEREMIAH, S. S., and BALIGA, P. R. (2011). Polymyxins: antimicrobial susceptibility concerns and therapeutic options. *Indian J. Med. Microbiol.*, 29(3), P.230.

[6] CADY, N. C., MCKEAN, K. A., BEHNKE, J., KUBEC, R., MOSIER, A. P., KASPER, S. H., BURZ, D. S. & MUSAH, R. A. (2012). Inhibition of biofilm formation, quorum sensing and infection in *Pseudomonas aeruginosa* by natural products-inspired organosulfur compounds. *PLoS One*, 7(6), P.e38492.

[7] CAI Y., CHAI D., WANG R., LIANG B., BAI N. (2012). Colistin resistance of *Acinetobacter baumannii*: clinical reports, mechanisms and antimicrobial strategies. *J. Antimicrob. Chemother.*, 67(7), pp.1607–1615.

[8] CARATTOLI A. (2001). Importance of integrons in the diffusion of resistance. *Vet. Res.*, 32(3-4), pp. 243-259.

[9] CHROMagar™ *Acinetobacter* (2018). The CHROM agar. [ONLINE] Available at: <http://www.chromagar.com/publication.php>. [Accessed 6 October 2018].

[10] CHROMagar™ COL-APSE (2018). Clinical Microbiology. [ONLINE] Available at: <http://www.chromagar.com/>. [Accessed 15 September 2018].

P7-2: Parallel outbreaks of carbapenem-resistant *Acinetobacter baumannii* in two long-term care facilities in Croatia

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Acinetobacter baumannii has become one of the most challenging agents of nosocomial infections in recent years; its relevance largely depends on a wide antimicrobial resistance, arising from a variety of intrinsic and acquired mechanisms. Of particular concern is resistance to β -lactam antibiotics, particularly carbapenems, which is often associated with the international clones clonal lineages 1-8.

Nine *A. baumannii* isolates with reduced susceptibility to carbapenems were isolated in Godan nursing home in Zagreb from February to March 2017, and four in April 2018 from Vitasan nursing home in Pula. Antibiotic susceptibilities were determined by broth microdilution. Genes encoding carbapenem-hydrolyzing oxacillinases, metallo- β -lactamases (MBLs), and ESBLs were investigated by PCR. Genotyping was performed by sequence group determination, PFGE and MLST. The incompatibility group of the plasmids was detected by multiplex PCR.

The strains were uniformly resistant to third and fourth generation cephalosporins, carbapenems, piperacillin/tazobactam, gentamicin and ciprofloxacin, but susceptible or intermediate susceptible to ampicillin/sulbactam, and susceptible to colistin. Four strains were found to be positive for *bla*_{OXA-23} and the rest for *bla*_{OXA-24/40}. PCR amplicons from four representative isolates were sequenced and revealed *bla*_{OXA-23} (strain 33980), *bla*_{OXA-72} (strains 5139-1, 41378 and 545-1) and *bla*_{OXA-66} (33980, 5139-1, 41378, and 545) allelic variants. *ISAbal* was found upstream of *bla*_{OXA-51} and *bla*_{OXA-23} gene. All isolates belonged to SG 1 (IC II). They contained *strAB*, *armA* and *aac(6')-II* aminoglycosides resistance genes, *sul 1* or *sul2* encoding resistance to sulphonamides, and *tetB* responsible for tetracycline resistance. All tested isolates from each center (eight from Zagreb and three from Pula) clustered by PFGE in one clone. Four strains were positive for group 2 plasmid. Comparison of PFGE patterns of the isolates from this study with those from 2013, originating from the same nursing home, revealed clonal relatedness (>80%). The study demonstrated spread of OXA-24-like positive strains not only in the clinical setting but also in long-term care facilities. The study found high genetic similarity of *A. baumannii* isolates from two centers located in distant geographic areas and genetic stability of isolates from different time periods.

P7-3: Molecular epidemiology of colistin-resistant *Acinetobacter baumannii* in Croatia

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Colistin, is a polypeptide antibiotic with a narrow spectrum of activity [1]. Mutations in the *phoPQ* and *pmrAB* TCS are most commonly implicated in colistin resistance in *Acinetobacter* spp, leading to the addition of L-arabinose or phosphoethanolamin to the lipid moiety, hiding the negative charge [2]. Recently colistin resistant *A. baumannii* was reported in Croatia.

In total 8 colistin resistant *A. baumannii* isolates were collected from 2017 to 2018 in University Hospital Osijek and County Hospital Pula in Croatia. Antibiotic susceptibility was determined by broth microdilution method according to CLSI[3]. The presence of carbapenemases of class A, B and D and extended-spectrum β -lactamase genes was explored by PCR [4]. The occurrence of the *ISAb₁* upstream of the *bla*_{OXA-51-like} or *bla*_{OXA-23-like} was determined by PCR mapping [5]. Conjugation and transformation experiments were performed as previously described [6]. Plasmid incompatibility groups were determined by PBRT [7]. Genotyping was performed by rep-PCR, determination of sequence groups and MLST.

All except two were isolated from infected patients with four isolates originating from patients with septicaemia. Three patients were previously treated with nebulized colistin. The isolates were uniformly resistant to ceftazidime, cefotaxime, ceftriaxone, cefepime, piperacillin/tazobactam, imipenem, meropenem, ciprofloxacin and colistin and all except one to ampicillin/sulbactam. Five out eight isolates were classified as pan-drug-resistant (PDR) as they were resistant to tigecycline as well. PCR was positive for *bla*_{OXA-23-like} in all tested isolates which was preceded by *ISAb₁*. All isolates were positive for group 2 plasmid encoding *Aci2* replicase gene, originally reported on pACICU plasmid. The isolates were classified into two clusters with four and two isolates respectively, whereas one isolate was a singleton. They all belonged to SG1 (ICII). Three different ST were reported: 1421, 195 and 1816.

Colistin resistance was associated only with OXA-23 CHDL although the dominant group of CHDL in Croatia is OXA-24 with OXA-72 as the only allelic variant reported so far. The study showed clonal spread of colistin resistant and OXA-23 positive organisms in the surgical ICU of the University Hospital Osijek in Croatia. Colistin resistance was combined with carbapenems and tigecycline resistance with no remaining therapeutic options left, resulting in the lethal outcome in half of the patients.

1. Li, J. *et al. Lancet Infect Dis* **6**, 589-601, doi:10.1016/s1473-3099(06)70580-1 (2006).

2-Bialvaei, A. Z. & Samadi Kafil, H. Colistin, mechanisms and prevalence of resistance. *Curr Med Res Opin* **31**, 707-721, doi:10.1185/03007995.2015.1018989 (2015).

3. Clinical and Laboratory Standards Institute 2013. 23rd Informational Supplement. Wayne, PA: CLSI, M100-S23.

4. Poirel, L., TR. Walsh, V. Cuveiller, and P. Nordman. 2011. *Diagn. Microbiol. Infect. Dis.* **70**:119-125.

5. Turton, JF., ME. Ward, N. Woodford, ME. Kaufman, R. Pike, DM. Livermore, and TL. Pitt. 2006. *FEMS Microbiol. Lett.* **258**:72-7.

6. Elwell, LP., and S. Falkow. 1986. In: Lorian V, ed. *Antibiotics in Laboratory Medicine*. 2nd ed. Baltimore MD: Williams and Wilkins, pp. 683-721.

7. Bertini, A., L. Poirel, P. Mugnier, J. Villa, P. Nordman, and A. Caratoli. 2010. *Antimicrobial Agents Chemother.* **54**:4168-4177.

P7-4: The *oxa23* gene carried by AbaR4 in carbapenem resistant *Acinetobacter baumannii* isolates recovered in an Iranian burn care center during an outbreak in 2012-2013

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Carbapenem resistance emerged in *Acinetobacter baumannii* shortly after the introduction and therapeutic use of carbapenems and disseminated globally. To date, two major clonal complexes, namely global clones 1 and 2 (GC1 and GC2), have mainly been responsible for the bulk of resistance. The emergence of carbapenem resistant *A. baumannii* (CRAB) has been a great concern and a particular threat in healthcare settings worldwide, as carbapenems are considered one of the last resort treatment options [1]. Carbapenem resistance in *A. baumannii* is mostly associated with horizontal acquisition of the oxacillinase genes, *oxa23*, *oxa24*, *oxa58* which have been commonly associated with mobile genetic elements including transposons and plasmids [2,3]. There are several reports from Iran pointing to the circulation of CRABs isolates carrying *oxa23* and *oxa24* [4]. However, the context of genes and the resistance mechanism have remained unknown. Here, a set of multiple antibiotic-resistant *A. baumannii* isolates belonging to global clone 1 (GC1), obtained from patients in a burn care center from Tehran, Iran, were examined. Following antibiotic susceptibility testing, PCRs were performed to detect antibiotic resistance genes and context of the *oxa23* gene. The sequence type (ST) of the isolates was determined using the Institut Pasteur Multi-locus Sequence Typing (MLST) scheme.

The 50 GC1 isolates causing outbreaks were recovered between 2012 and 2013 from patients admitted to one of the wards in a single center that provides healthcare services for burn patients. All the isolates were resistant to carbapenems, contained *oxa23* and *oxa24*. However, they did not generate an amplicon for the *comM* gene indicating either the gene is not present or interrupted. Further analysis showed that all 50 isolates carried the *oxa23* within Tn2006, located in the AbaR4 resistance island, which was found in the *comM* gene. The isolates belongs to ST328 which is a single locus variant (SLV) of the GC1 sequence type, ST18. The genome of a representative GC1 isolate carrying *oxa23* and *oxa24* was sequenced using Illumina HiSeq, assembled using the SPAdes program and analysis of the genome identified *oxa24* on a plasmid.

For the first time, this study determines the context of the *oxa23* gene, as the most encountered oxacillinase gene, in a set of CRAB strains recovered in Iran. It also provides evidence for the prolonged outbreaks caused by CRABs isolates carrying *oxa23* and *oxa24*. Further analysis will be needed to determine the location of *oxa24* in other isolates.

[1] Holt K, Kenyon JJ, Hamidian M, Schultz MB, Pickard DJ, Dougan G, Hall R. Five decades of genome evolution in the globally distributed, extensively antibiotic-resistant *Acinetobacter baumannii* global clone 1. *Microb Genom*. 2016;2:e000052.

[2] Evans BA, Amyes SG. OXA β -lactamases. *Clin Microbiol Rev*. 2014;27:241-63.

[3] Nigro SJ, Hall RM. Structure and context of *Acinetobacter* transposons carrying the *oxa23* carbapenemase gene. *J Antimicrob Chemother*. 2016;71:1135-47.

[4] Peymani A, Higgins PG, Nahaei MR, Farajnia S, Seifert H. Characterization and clonal dissemination of OXA-23-producing *Acinetobacter baumannii* in Tabriz, northwest Iran. *Int J Antimicrob Agents*. 2012;39:526-8.

P7-5: Molecular characterization of MDR clinical isolates of *A. baumannii* from three neighbouring countries in south-eastern Europe

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In the last decade, *Acinetobacter baumannii* has emerged as a major relevant nosocomial pathogen in the most European countries. Multidrug-resistant (MDR) clinical isolates represent a major problem in the epidemiological and therapeutic sense and challenge for clinicians. Surveillance of carbapenem- and MDR resistant isolates of *A. baumannii* in Croatia is continuously monitored since in the last decade the percentage of carbapenem-resistant isolates reached 97%. Along Croatia, neighbouring countries Bosnia and Herzegovina and Serbia also recorded a significant increase in the incidence and prevalence of carbapenem-resistant isolates of *A. baumannii*. The aim of this pilot study is to compare the genotype resemblance and resistance mechanism of MDR clinical isolates of *A. baumannii* in region of south-eastern Europe.

In total, 12 clinical isolates of carbapenem-resistant *A. baumannii* were collected from three different hospitals in neighbouring countries: Croatia, Bosnia and Herzegovina, and Serbia. Four isolates originated from University Hospital of Split, Croatia and were isolated from the tracheal and bronchoalveolar aspirates of patients from adult and paediatric Intensive Care Units in different outbreaks periods from 2009-2018. Two isolates were collected from University Hospital Mostar, Bosnia and Herzegovina in the beginning of 2018. Six isolates came from different wards of Clinical Centre of Vojvodina, one hospital in university-affiliated medical centre Novi Sad, Serbia and were collected from blood cultures during 2017 and 2018.

All collected isolates shared high level of resistance to carbapenems with MIC >32mg/L to both imipenem and meropenem. Beside the carbapenem-resistance, isolates were uniformly resistant to gentamicin and ciprofloxacin, but susceptible to colistin. The relatedness of collected *A. baumannii* isolates was assessed by using pulsed-field gel electrophoresis (PFGE) and displayed diversity of genotyping profiles. The multiplex PCR confirmed the presence of *bla*_{OXA-40-like} genes in half (6/12) of the collected isolates from neighbouring countries, beside the presence of *bla*_{OXA-23} gene. These are the first results of a pilot study on MDR clinical isolates of *A. baumannii* originating from three neighbouring countries in south-eastern Europe.

P7-6: Surface Polysaccharide Variation in the *Acinetobacter baumannii* ST25 Clonal Lineage

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The global expansion of antibiotic resistant *Acinetobacter baumannii* lineages has prompted investigation into effective epidemiological markers for tracing local and global spread. Sequence diversity observed at the genomic K locus (KL) for synthesis of the highly variable capsular polysaccharide (CPS) and the OC locus (OCL) for synthesis of the outer-core component of the lipooligosaccharide have previously proven valuable in epidemiological studies of the major global clonal lineages [1]. However, little is known about these regions in the globally prominent and increasingly antibiotic resistant sequence type 25 (ST25). To investigate the extent of diversity at the K and OC loci in ST25 genomes, 3,417 total genome sequences were downloaded from the NCBI Whole Genome Sequence (WGS) and GenBank databases. Multilocus sequence typing (MLST) revealed 81 genome sequences belonging to ST25, though 20 were removed from analysis due to assembly quality. The remaining genomes were assessed for KL and OCL types using Kaptive [2] and our recently developed database of KL and OCL reference sequences. A total of 19 KL and 5 OCL gene clusters were identified in the set, with 5 KL and 1 OCL being novel gene cluster arrangements annotated according to the established nomenclature [1]. Of the 61 genomes examined, KL14 was the most common K type and OCL5 was the predominant OCL type. Though extensive variation of both KL and OCL regions was observed, specific KL were found with a variety of OCL types. For example, KL37 was found with OCL5, OCL6, OCL7 and OCL10. By using the combination of KL and OCL, it is possible to map global distribution patterns and delineate sub-lineages within the clone. The results obtained indicated that the KL14-OCL6 combination was found globally, whereas other locus combinations were restricted to particular geographical regions. Tracing K and OC types and their evolution enables more thorough discrimination between strains belonging to the same clonal lineage, and establishes the combination of KL and OCL as a valuable epidemiological marker.

1. J. Kenyon, R.M. Hall. Variation in the Complex Carbohydrate Biosynthesis Loci of *Acinetobacter baumannii* Genomes. PLOS ONE 8(4), (2013). e62160.
2. R.R. Wick, E. Heinz, K. Holt, & K.L. Wyres. Kaptive Web: User-Friendly Capsule and Lipopolysaccharide Serotype Prediction for Klebsiella Genomes. Journal of clinical microbiology, 56(6), (2018). e00197-18.

P7-7: One-year monitoring study of epidemiology and antibiotic resistance of *Acinetobacter baumannii* isolates in one hospital in Northern Italy

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Acinetobacter baumannii (AB) has become a difficult to treat pathogen worldwide due to its ability to acquire multi-drug resistance (MDR), particularly to carbapenems and aminoglycosides. Our study investigated epidemiology and antibiotic resistance of AB strains collected during 2018 at Lecco Hospital, Northern Italy. A total of 30 consecutive non-repeated AB isolates showing a MDR profile were collected in a one-year period from both inpatients (n=18, belonging to eight wards) and outpatients (n=3, from the community setting; n=2, from Long-Term Care Facilities; n=1, from a Geriatric Institution; n=1; from a territorial Hospital). Species identification and susceptibility profiles were obtained by MALDI-TOF MS, Vitek-2 System and MICRONAUT-S MDR. Interpretation of susceptibility profiles was carried out according to current EUCAST criteria. Resistance determinants for carbapenemases, aminoglycosides and fluoroquinolones were identified by PCR. Typing was performed by Pulsed-Field Gel Electrophoresis (PFGE) and Multilocus Sequence Typing (MLST). The specimens were from respiratory secretions (n=9), followed by urine (n=6), rectal/nasal/pharyngeal swabs (n=6), blood/venous catheter tips (n=3), surgical wounds (n=2) and other sources (n=4). Twenty-four (80%) isolates were resistant to meropenem (MEM), imipenem (IPM), fluoroquinolones and trimethoprim-sulfamethoxazole (SXT), while resistance to aminoglycosides was detected in 23 isolates (76.6%). Twelve isolates collected from blood/catheter tips of ICU inpatients and respiratory tract specimens of ICU, Neurosurgery, Neuro-ICU and the Medicine Units were deeper characterized. Concerning the above isolates, the antibiotic resistance rate was 91.6% (n=11/12) against MEM, IPM, ciprofloxacin, levofloxacin, SXT. The 83% of the isolates (n=10/12) were tigecycline and 25% (n=3/12) colistin resistant. Strains were all positive for *IsAba-blaOXA-23*, while 11 and 10 isolates were positive for *aac(6')-Ibcr* and *ArmA* determinants, respectively. Six PFGE patterns (A-G) belonging to two Clonal Groups (CG2 and a new CG) were identified. The three PFGE profiles A [n=1, Neuro-ICU; n=1, ICU], C (n=4, ICU; n=1, Neuro-ICU) and D (n=2, neuro-ICU) were indicative of clonal micro-diffusion events within ICU wards. The present results highlight the circulation of strains carrying *blaOXA23* and the co-carriage of *ArmA* and *aac(6')-Ib-cr* genes among MDR AB isolates in the critical care setting of Lecco Hospital, Northern Italy.

P7-8: Putting gender perspective on the agenda of *Acinetobacter baumannii* research

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During the last years international organizations such as the World Health Organization and an increasing number of scientific publications highlight the need of taking into account sex and gender factors in the area of the infectious diseases as they affect directly to the infectious process in aspects as vulnerability, exposure and the immune response. There is little information about how microorganisms, including *Acinetobacter* spp., affect women's health differently as male models have been the reference up to now. Gender inequalities in research concern the presence of women as models of study and as researchers, so that the objectives of this work were: A) to analyse the presence of female researchers in the *Acinetobacter* symposium since 2000 year to the present in terms of representation in the meeting and B) to study retrospectively, taking into account the sex of the patients, results from the *Acinetobacter baumannii* Research Group (Bilbao, Spain) since 1999 in collaboration with hospitals in Bilbao (Spain) and Cochabamba (Bolivia).

Although female attendance has been ranging from a 45 to 50%, the presence of female researchers in the scientific committee and chairs decreased from the year 2000 to 2017 from 40% to 18% and from 33% to 19% respectively. Concerning keynote talks, there is a mean of 25% given by women. Women as first authors in oral communications increased from a 20% to a 42% in 2017.

From Hospital Santa Marina, Bilbao, we studied 185 patients (133 male, 52 female). We observed that the number of infections in women increased during the period of study while infections in men decreased. There were also differences in the type of infection being respiratory tract infections more frequent in men while in women the most prevalent were pressure ulcer infections.

From Bolivia, we studied 190 patients (109 male, 81 female) from Hospital Gastroenterológico Boliviano Japonés, Hospital Materno Infantil and from Hospital Viedma. Among infant patients, sepsis was the most prevalent diagnostic in female and pneumonia in male. Among adults wound infections were the most frequent in both sexes, although more prevalent in women.

In conclusion, more studies with gender perspective are needed as the results obtained indicate that there are differences between men and women concerning infections caused by *Acinetobacter baumannii*. Underrepresentation of female researchers in the *Acinetobacter* symposiums could be due to gender inequalities.

P7-9: *Acinetobacter baumannii* and One Health – Epidemiology and antibiotic resistance profiling of isolates from Norwegian chicken farms

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Acinetobacter baumannii is included in the ESKAPE pathogens, the six leading causes of nosocomial infections in the world, and carbapenem-resistant isolates (MDR) are classified as WHO priority 1 critical pathogens for research on novel antimicrobials and new treatment options. The bacterium can also be found in many different environmental reservoirs outside of hospitals. Thus, increasing the understanding of the epidemiology of *A. baumannii* and possible phylogenetic links between environmental, animal, and clinical human isolates is potentially of high importance – and constitutes a field, which appears to be underexplored.

In the current study we use Multilocus Sequence Typing (MLST; Pasteur scheme) to reconstruct the phylogeny of 178 *A. baumannii* isolates sampled from chicken farms across Norway during 2016 (sock samples). We present a phylogenetic comparison of these isolates to international clinical and environmental *A. baumannii* strains. A selection of clinical isolates collected in Norwegian hospitals in the period 2016-2019 will also be included in the study. Furthermore, the drug susceptibility profile of the chicken farm isolates was also assessed, using a disk diffusion assay. In connection with drug susceptibility testing, the chicken farm isolates are being tested for possible phase variation phenotypes through investigation of colony morphology under oblique light. Selected strains from the study will be chosen for whole genome sequencing.

P7-10: Molecular Epidemiology of Carbapenem resistant *Acinetobacter baumannii* Isolated from Khartoum State, Sudan

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Acinetobacter baumannii has emerged as an important multi-drug resistant organism that is associated with nosocomial infections worldwide [1]. This study aimed to explore the molecular epidemiology and antimicrobial resistance mechanisms using whole-genome sequencing (WGS) of carbapenem resistant *A. baumannii* (CRAb) isolated from patients at Khartoum State, Sudan. Twenty two non-duplicate CRAb were collected between October 2016 and February 2017, from a variety of clinical specimens obtained from patients in two Hospitals at Khartoum State.

Species identification and carbapenem resistance mechanisms were investigated initially using phenotypic and PCR detection methods using different multiplex PCRs for *gyrB*, OXA group (OXA-23,-40,-51,-58,-143 and -235), and in-house multiplex PCRs to detect genes encoding NDM, IMP, VIM, GIM, KPC and GES [2]. The isolates were further characterized by WGS (Illumina MiSeq), and the molecular epidemiological characters and resistance mechanisms were identified [3].

All isolates were phenotypically carbapenem-resistant and harboured several β -lactamase genes. The gene encoding the acquired OXA-23 was detected in 21/22 (95%) of the isolates, three of which co-harboured OXA-58. Other carbapenemases detected were: NDM-1 4/22 (18%) and GES-11 2/22 (9%). TEM-1D was detected in 20/22 (90%) isolates. One isolate had *ISAb_a1* upstream of *bla*_{OXA-51}, as well as genes encoding OXA-1 and CTX-M-15. Furthermore many other acquired resistance genes were detected conferring resistance to aminoglycosides, macrolides, fluoroquinolones, phenicols, tetracyclines, sulphonamides, and trimethoprim. 19/22 (86%) of the isolates clustered with IC2 and had the intrinsic *bla*_{OXA-66}. Two isolates clustered with different ICs harboured the intrinsic OXA genes: IC1 with *bla*_{OXA-69}, and IC5 with *bla*_{OXA-91}. The sporadic isolate had *bla*_{OXA-51}. Molecular typing based on cgMLST revealed two transmission clusters containing isolates from two hospitals, with 12, and 3 isolates. In conclusion, our CRAb isolates mainly belonged to IC2 and constitute a prominent problem in our local setting. The OXA-23 producing isolates frequently possess other resistance genes coding for NDM-1, GES-11 and OXA-58. For prevention, screening, surveillance and infection control must be enhanced.

[1] Gonzalez-Villoria *et al.* J Pathog, 2016;7318075.

[2] Higgins *et al.* Antimicrob Agents Chemother, 2013;57:2121-26.

[3] Higgins *et al.* PLoS One. 2017;1–13.

P7-12: The distribution and antibiotic susceptibility patterns of Non-*baumannii* *Acinetobacter* species from a tertiary hospital in Korea

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The role and importance of non-*baumannii* *Acinetobacter* species in human infection have been increasingly reported with technological advances in recent [1,2]. In this study, we investigated the prevalence of non-*baumannii* *Acinetobacter* spp. and the pattern of antibiotics susceptibility in a tertiary hospital, Korea.

From January 2012 to December 2018, we collected the microbiology laboratory data of non-*baumannii* *Acinetobacter* isolated from patients hospitalized at a tertiary university hospital, Korea. Identification and antibiotic susceptibility test (AST) were done by VITEK II (bioMérieux, France).

Collected non-*baumannii* *Acinetobacters* were 374 isolates. *A. Iwoffii* was leading one (38.7%), followed by *A. junii* (13.1%), *A. ursingii* (10.2%), and *A. haemolyticus* (6.1%). The incidence of all species did not change significantly according to the year. Sputum and urine accounted for the majority of specimens (46.5%, 34.2%, respectively). *A. lowffii* was also identified from blood (4.7%). *A. lowffii* showed the considerable resistance rates of carbapenems (15.6%). Resistant *A. lowffii* isolates against colistin and tigecycline were 8 (3.1%) and 4 (1.6%), respectively. And the isolates of colistin resistant *A. junii*, *A. ursingii*, and *haemolyticus* were 16 (32.7%), 5 (13.2%), and 4 (17.4%).

In Korea, the prevalence of non-*baumannii* *Acinetobacter* cannot be ignored. Also, Resistant isolates against colistin and tigecycline were identified. Considering the results, in the future, the increase of extensively-drug resistant and pan-drug resistant non-*baumannii* *Acinetobacter* can cause the significant health problem worldwide. Therefore, the various researches for non-*baumannii* *Acinetobacter* including antibiotic resistance-related mechanism are needed.

[1] A. Al Atouni, M.-L. Joly-Guillou, M. Hamze, M. Kempf. Reservoirs of non-*baumannii* *Acinetobacter* species. *Front Microbiol.* **7** (2016)

[2] D. H. Kim, K. S. Ko, AbaR-type genomic islands in non-*baumannii* *Acinetobacter* species isolates from South Korea, *Antimicrob Agents Chemother.* **59** (2015), 5824-5926

P7-13: Prevalence report of *Acinetobacter baumannii* in pediatric patients from Lima, Peru

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Acinetobacter baumannii is an opportunistic gram-negative pathogen, recognized for causing a wide variety of nosocomial infections, being nosocomial pneumonia associated with greater mortality [1-2]. In Peru, pneumonia continues to be the leading cause of premature mortality, with an incidence of acute respiratory infections of 4143.07 patients per 10,000 inhabitants due to acute respiratory infection by mid-2018; however, information on nosocomial pneumonia in the pediatric population and the pathogens involved is still limited [3]. In this study, the prevalence of *A. baumannii* in children aged less than one year hospitalized for more than 2 days with a probable diagnosis of Whooping Cough was determined. The presence of *A. baumannii* was detected in 19.05% (48/252) of the nasopharyngeal swab samples by Real Time PCR of the OXA-51 gene. Children aged from 29 days to 3 months were the most affected, with a prevalence of 52.1% (25/48), followed by 25% (12/48) in children aging 3 to 5 months.

The most frequent symptoms in the positive cases were: paroxysmal cough (85.42%, 41/48), respiratory difficulty (79.17%, 38/48), redness (68.75%, 33/48), cyanosis (62.5%, 30/48).) and difficulty in breastfeeding (58.33%, 28/48). It was found that 56.25% (27/48) of the positive cases showed coinfection with *Bordetella pertussis*, 4.17% (2/48) with *Mycoplasma pneumonia* and Respiratory syncytial virus type A, and 2.08% (1/48) with parainfluenza virus type I.

It is concluded that it is necessary to carry out more studies on the role played by *A. baumannii* in hospitals in order to determine its frequency and the implications in the clinical and therapeutic management of the pediatric patient.

[1]Bergogne-Bérézin E, Towner KJ. *Acinetobacter* spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features. Clin Microbiol Rev [Internet]. 1996 Apr [cited 2018 Nov 16];9(2):148–65. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/8964033>

[2] Dijkshoorn L, Nemec A, Seifert H. An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*. Nat Rev Microbiol [Internet]. 2007 Dec 1 [cited 2018 Sep 20];5(12):939–51. Available from: <http://www.nature.com/articles/nrmicro1789>

[3]Ministerio de Salud. Situación de salud de IRAS ., 2018 [cited 2019 May 10];24. Available from: <http://www.dge.gob.pe/portal/docs/tools/02.pdf>

P8-1: Chalcones as potential inhibitors of polymicrobial biofilm produced by MDR *Acinetobacter baumannii* wound isolates

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Acinetobacter baumannii has a tremendous ability of biofilm formation under various different conditions, which in turn enables its survival in harsh, desiccated environments for a long time. Furthermore, this pathogen ordinarily exists in the form of polymicrobial biofilm communities, interacting with other microbes, which additionally complicates the treatment of these structures. The skin of hospitalized patients is a common place where *A. baumannii* can be found in biofilm populations co-existing with pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Candida* spp. Here we examined the biofilm production of MDR *A. baumannii* hospital wound isolates by microtiter plate assay and proved that substantial amounts of biofilm can be produced under various conditions, including the broad range of temperatures (25-45 °C), various growth media, or after the treatment with antiseptics such as boric acid and ethacridine lactate. According to results, the presence of glucose in growth media mostly contributed to the level of biofilm production, whereas boric acid was most successful in its inhibition. Then we tested the inhibitory potential of two synthesized chalcones on polymicrobial biofilm production by MDR *A. baumannii* in combination with each of the previously mentioned common skin inhabitants. Chalcones were selected based on previous results, as successful monomicrobial *A. baumannii* biofilm inhibitors, comparable to action of meropenem and ciprofloxacin, and applied in subinhibitory concentrations (35 µg/mL and 70 µg/mL). The results suggest significant inhibitory potential on biofilm production of *A. baumannii* + *S. aureus* and *A. baumannii* + *Candida* spp. combinations (inhibition by 30-40%). Diversely, the formation of biofilm was significantly stimulated in treated *A. baumannii* + *P. aeruginosa* combinations. We conclude that tested *A. baumannii* strains have an ability to form biofilm under the broad range of various conditions, whereas two examined synthesized chalcones can substantially inhibit the polymicrobial biofilm formation of certain combinations which involve MDR *A. baumannii*.

P8-2: Novel Non- Lytic *Acinetobacter* Phage AbDs1: Mechanism of Infection and Manipulation of its Genome to Deliver Antibacterial Proteins or Peptides into Multi Drug Resistant *Acinetobacter baumannii* Strains

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Acinetobacter baumannii has become a major healthcare threat, responsible for both hospital and community acquired infections [1]. In the last 30 years, *A. baumannii* has evolved from being an underestimated microorganism to one of the most important agents causing nosocomial infections including pneumonia, meningitis, septicaemia, and urinary tract infections contributing to epidemic outbreaks in hospitals [2]. *A. baumannii* has natural competence to acquire genetic material from environment or through lateral gene transfer. This extraordinary ability of the strain resulted in evolution of multidrug-resistant (MDR) and extremely drug-resistant (XDR) strains [3].

We have isolated non-lytic phage AbDs1 from *Acinetobacter baumannii* DS002, isolated from organophosphate pesticide polluted soil. The genome of AbDs1 has just three open reading frames (ORFs). One of them is *repA*, which helps in replication of the phage genome. The other two ORFs, *orf113* and *orf96* code for coat proteins [4]. Our pull-down assays have shown interaction of coat protein Orf96, with outer membrane protein, OmpA. Even the OmpA null mutant of *A. baumannii* failed to serve as host for AbDs1. The biochemical and genetic evidences gathered in this study suggests requirement of OmpA for gaining entry of AbDs1 into *A. baumannii* DS002. OmpA is conserved across *A. baumannii* strains. Even the MDR strains of *A. baumannii* contain highly conserved OmpA, suggesting a possibility of AbDs1 using MDR strains of *A. baumannii* as host. Thus, the usage of AbDs1 as vehicle to deliver antimicrobial peptides is suggested.

[1] Visca P, Seifert H and Towner KJ. *Acinetobacter* infection-an emerging threat to human health. *IUBMB Life*. (2011), 1048–1054.

[2] Alsan M and Klompas M. *Acinetobacter baumannii*: An Emerging and Important Pathogen. *J Clin Outcomes Manag*. (2010), 363-369.

[3] Traglia GM, Place K, Dotto C, Fernandez JS, Montana S, Bahiense CDS, Soler-Bistue A, Iriarte A, Perez F, Tolmasky ME, Bonomo RA, Melano RG and Ramirez MS. Interspecies DNA acquisition by a naturally competent *Acinetobacter baumannii* strain. *Int J Antimicrob Agents*, **53** (2019), 483-490.

[4] Longkumer T, Kamireddy S, Muthyala VR, Akbarpasha S, Pitchika GK, Kodetham G, Ayaluru M, Siddavattam D. *Acinetobacter* phage genome is similar to Sphinx 2.36, the circular DNA copurified with TSE infected particles. *Scientific reports* (2013).

P8-3: Characterization of Blp1 adhesin as vaccine candidate against multidrug-resistant *Acinetobacter baumannii*

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The multidrug-resistant (MDR) *Acinetobacter baumannii* is difficult to treat infection agent, causing nosocomial infections world-wide [1]. Therefore, it has been considered as top priority pathogen, for which new drugs and therapeutic options are urgently needed [2]. Several vaccine candidates against infections caused by *A. baumannii* have been proposed [3]. *A. baumannii* expresses a capsular layer, which covers the bacterial surface and protects it from the host immune system. To overcome this, large extracellular adhesins, which might penetrate the capsule layer, can be adapted as new vaccine candidates. The recently described *A. baumannii* Blp1 adhesin could represent a promising antigen for such applications [4].

We demonstrated, that markerless *blp1* gene deletion mutant showed decreased virulence in nematode and mice infection models compared to wild type *A. baumannii* strain, indicating its importance during infection *in vivo*. Furthermore, we have tested over 100 clinical *A. baumannii* isolates, belonging to the globally spread international clonal lineages I and II (IC I, IC II), and confirmed the presence of Blp1 coding gene in all tested strains. Importantly, the sequence at the very end of Blp1 protein C-terminus is fully conserved. We have used the C-terminus of Blp1 protein for the mice vaccinations. Mice, immunized with C-terminus of Blp1 protein or Blp1-specific antisera, demonstrated the higher survival rate after challenging with *A. baumannii* comparing to the control group using both active and passive immunizations. Moreover, the opsonophagocytic killing assay demonstrated an effective neutralization of two clinical *A. baumannii* strains, belonging to IC I and IC II. In conclusion, the results represent Blp1 protein as a promising target for vaccine development against *A. baumannii* infections.

[1] A. Giammanco, C. Calà, T. Fasciana, M. J. Dowzicky. Global Assessment of the Activity of Tigecycline against Multidrug-Resistant Gram-Negative Pathogens between 2004 and 2014 as Part of the Tigecycline Evaluation and Surveillance Trial. *mSphere* **2** (2017), pii: e00310-16

[2] B. Isler, Y. Doi, R. A. Bonomo, D. L. Paterson. New Treatment Options Against Carbapenem-Resistant *Acinetobacter baumannii* Infections. *Antimicrob Agents Chemother.* (2018), pii: AAC.01110-18.

[3] T. A. Ahmad, D. M. Tawfik, S. A. Sheweita, M. Haroun, L. H. El-Sayed. Development of immunization trials against *Acinetobacter baumannii*. *Trials in Vaccinology* **5** (2016); 53-60

[4] E. Gregorio, M. Del Franco, M. Martinucci, E. Roscetto, R. Zarrilli, P.P. Di Nocera. Biofilm-associated proteins: news from *Acinetobacter*. *BMC Genomics* **16** (2015), 933.

P8-4: Antibacterial activity of colloidal silver against multidrug-resistant *Acinetobacter baumannii*

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Treatment of multidrug-resistant (MDR) Gram-negative bacilli represents a challenge for clinicians and public health authorities. Due to the emergence of resistance to a wide variety of antibiotics new alternative therapies are needed. Silver has been used to treat bacterial infections since antiquity due to its known antimicrobial properties. The objective of this study was to evaluate *in vitro* the activity of colloidal silver against MDR *Acinetobacter baumannii*.

Reference and clinical strains of *A. baumannii* (n=45) were used. All strains were grown in a Mueller-Hinton Broth (MHB) at 37°C for 20-24 h. Minimal inhibitory concentration (MIC) of colloidal silver was determined for all strains by using microdilution assay. To monitor the bactericidal activity of the colloidal silver, time-kill curve assays of representative reference and MDR clinical strains of *A. baumannii* (ATCC 17978 and #11) were performed on MHB at colloidal silver concentrations of 0.5x, 1x and 2x MIC with starting bacterial inoculum of 1x10⁶ CFU/mL. To identify the colloidal silver mechanism of action, membrane permeation was analyzed using fluorescence impermeant-indicator. Moreover, bacterial reactive oxygen species (ROS) production was measured at 6, 20 and 24 hours at colloidal silver concentrations of 0.25x, 0.5x and 1x MIC.

Colloidal silver MIC₉₀ was 4 mg/L for the collection of *A. baumannii* strains. Colloidal silver showed bactericidal activity against representative reference and clinical strains of *A. baumannii*. Colloidal silver was bactericidal at 1x and 2x MIC at 24 hours of incubation. Permeabilization assays showed that colloidal silver did not affect the membrane permeabilization of reference and MDR strains of *A. baumannii*. Furthermore, analysis of ROS production revealed that colloidal silver increased significantly the ROS production in these kinds of pathogens at 24 hours of incubation.

Altogether, these results suggest that colloidal silver could be an effective treatment for infections caused by MDR *A. baumannii*.

P8-5: Tamoxifen metabolites as repurposing drug against multidrug-resistant *Acinetobacter baumannii*

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The development of new strategic antimicrobial therapeutic approaches, like the repurposing of drugs as monotherapy or in combination with clinically relevant antibiotics, has become an urgent need. Previously, we reported that tamoxifen, a selective estrogen receptor antagonist used for treatment of breast cancer, presented therapeutic efficacy against *Acinetobacter baumannii* *in vivo*, without showing *in vitro* bactericidal activity. We hypothesized that the *in vivo* tamoxifen antimicrobial efficacy results from its three major metabolites: desmethyltamoxifen, hydroxytamoxifen and endoxifen, released by cytochrome P450 mediated metabolism. The objective of this study was to evaluate the *in vitro* activity of the tamoxifen metabolites together against MDR *A. baumannii*.

Reference and clinical strains of *A. baumannii* (n=100) were used. Minimal inhibitory concentration (MIC) of tamoxifen metabolites was determined for all strains by using microdilution assay. To monitor the antibacterial activity of tamoxifen metabolites, time-kill curve assays of reference and MDR clinical strains of *A. baumannii* (ATCC 17978 and Ab186) were performed at tamoxifen metabolites concentrations of 1x, 2x and 4x MIC with starting bacterial inoculum of 1×10^6 CFU/mL. To identify the mechanism of action of tamoxifen metabolites, membrane permeabilization by fluorescence impermeant indicator Ethidium Homodimer-1 and outer membrane proteins (OMPs) profiles by SDS-PAGE were determined.

Tamoxifen metabolites MIC₉₀ was 16 mg/L for the collection of *A. baumannii* strains. Tamoxifen metabolites showed bactericidal activity against reference and clinical strains of *A. baumannii*. Tamoxifen metabolites were bactericidal at 2x and 4x MIC at 8 h of incubation, and at 4x MIC at 24 h of incubation. Permeabilization assays showed that MDR strains of *A. baumannii* treated with tamoxifen metabolites during 24 h presented higher membrane permeabilization than the reference strains. Furthermore, analysis of OMPs profile after 24 h of incubation with tamoxifen metabolites did not present changes in reference and MDR strains of *A. baumannii*, indicating that tamoxifen metabolites did not affect their OMP expression.

Altogether, these results showed that tamoxifen metabolites presented *in vitro* antibacterial activity against MDR *A. baumannii*. This activity could explain the *in vivo* therapeutic efficacy of tamoxifen against infections by this pathogen.

P8-6: Antimicrobial activity of essential oils and synergy with colistin against colistin-susceptible and colistin-resistant *Acinetobacter baumannii* strains

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Background: *A. baumannii* has a high capacity to develop or acquire new antimicrobial resistance mechanisms, therefore the search for new therapeutic alternatives is necessary. One option is the use of combined therapies between antibiotics and adjuvants. Essential oils (EOs) are aromatic oily liquids consist of up to 100 secondary metabolites. Some EOs and their constituents may have some antibacterial, antifungal or antiviral activity. The aim of the study was to determine in *A. baumannii* which are the best EOs candidates to use as antibiotic adjuvants and which are the best antibiotic/adjuvant combinations *in vitro* and *in vivo*.

Methods: Disc diffusion and determination of MICs assays were used to study the antimicrobial activity of 10 EOs against two representative strains of *A. baumannii* (ATCC 17978 and ATCC 19606). The three most active EOs were deeply studied in more strains. The activity of this EOs in combination with antibiotics (imipenem, meropenem, colistin, tigecycline, ceftazidime and amikacin) were assessed. Checkerboard method was used and FIC_{index} was calculated. Finally, efficacy of combined therapy was studied in an *in vivo* model of *Galleria mellonella*.

Results: EOs of clove, thyme and eucalyptus (MIC 512 mg/L) were the most active against *A. baumannii* strains. Synergy was observed with EOs of thyme and clove in combination with colistin. No synergy was observed with EO of eucalyptus and/or other antibiotics.

Two colistin-resistant derivatives of ATCC 19606 (ATCC 19606*pmrB* and ATCC 19606 Δ *pxC*) and two clinical isogenic pair of colistin-susceptible/colistin-resistant strains (AB248/ AB249*pmrB* and ABRIM/ ABRIM*pmrB*) were studied. In ATCC 19606*pmrB*, colistin MICs decreased 16-fold in combination with 128 mg/L of EOs of thyme or clove. In ATCC 19606 Δ *pxC*, colistin MICs decreased 16- and 32-fold in combination with 128 mg/L of EO of thyme and clove, respectively. In the pair AB248/ AB249*pmrB*, colistin MICs decreased 16- and 128-fold in combination with 128 mg/L of EO of thyme, respectively, while colistin MICs decreased 16- and 64-fold in combination with 128 mg/L of EO of clove. In the pair ABRIM/ ABRIM*pmrB*, colistin MICs decreased 4- and 128-fold in combination with 128 mg/L of EO of thyme, respectively, while both strains decreased 8-fold in combination with 128 g/L of EO of clove. Preliminary *in vivo* assays showed better efficacy of combination therapy compared to monotherapy in colistin susceptible and colistin resistant strains.

P8-7: Mechanism based study and chemical characterization of carbapenem resistant strain of *Acinetobacter baumannii* treated with effective concentrations of disinfectant

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Acinetobacter baumannii is an opportunistic pathogen associated with hospital-acquired infections [1]. The ongoing increase in incidence, largely associated with infected combat troops coming back from conflict zones, coupled with a dramatic increase in the incidence of multidrug-resistant (MDR) strains, has altogether raised the profile of this emerging opportunistic pathogen [2]. To remove or reduce the bacteria various disinfectants, such as ClO_2 , CHX, and H_2O_2 , are increasingly being used in the hospital setup. The high concentration of disinfectant had also showed toxicity and increase cost of hospital cleaning, therefore it is important to find the effective concentration of these disinfectants. We have tested different concentrations of these disinfectants against six strains of *A. baumannii* that includes 5 resistant strains (RS) 307, 122, 7434, 10953, 6694 and 1 sensitive strain ATCC 19606 by monitoring the growth kinetics, disc diffusion assay, MIC determination, Reactive oxygen species, Lipid peroxidation assay, protein carbonyl, Fourier-Transform infrared spectroscopy and Flow Cytometry assay. All the experiments were performed in triplicates and the data expressed is Mean \pm SEM of at least three values ($n = 3$). Result confirms that Growth kinetics showed 10 mM ClO_2 , 32 $\mu\text{g/ml}$ CHX, and 2.5% H_2O_2 were significantly inhibits the strains of *A. baumannii*. MIC value were found to be 12.5 mM of ClO_2 , 2 $\mu\text{g/ml}$ for CHX with 25.64% of its cell viability, and 0.1% H_2O_2 produced 20% viability. It was also seen that ROS generation significantly increase after treatment with 10 mM ClO_2 , 32 $\mu\text{g/ml}$ CHX, and 2.5% H_2O_2 (5 fold, 80%, 12.7 fold increased, respectively). Similarly, treatment also leads to alteration in the LPO, but no carbonyl content observed. SDS-PAGE result also showed the alteration in the banding pattern as compared to untreated sample. FTIR results shows difference in the spectra of untreated bacterial strain as compared to the treated bacterial strain. Together with this, results of Flow cytometry assay confirms that more dead cells observed in the disinfectant treated samples. With these results we can conclude that disinfectants are effective against *A. baumannii*, and cost effective and less harmful for the hospital patients as well as for workers and staffs.

1. Biswas, D., M. Tiwari, and V. Tiwari, *Comparative mechanism based study on disinfectants against multidrug-resistant Acinetobacter baumannii*. J Cell Biochem, 2018. **119**(12): p. 10314-10326.
2. Verma, P., et al., *In-silico interaction studies suggest RND efflux pump mediates polymyxin resistance in Acinetobacter baumannii*. J Biomol Struct Dyn, 2019. **37**(1): p. 95-103.