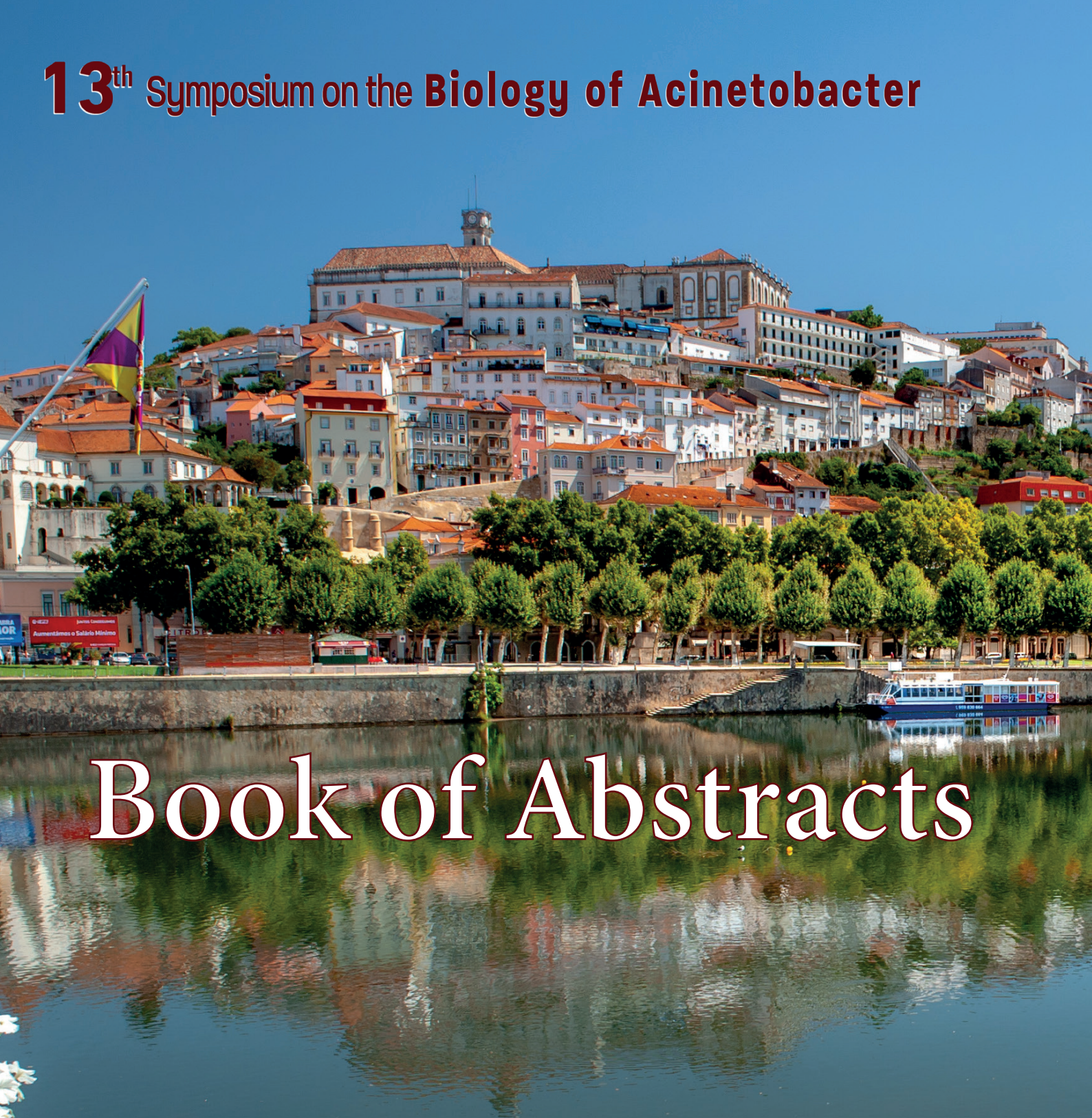
13th Symposium on the Biology of Acinetobacter



Book of Abstracts

21st - 23rd june 2023
Coimbra - Portugal

Auditorium of the Rectory of the University of Coimbra

Federation of European Microbiological Societies

1290 UNIVERSIDADE D COIMBRA

1290 FACULDADE DE FARMÁCIA UNIVERSIDADE D COIMBRA

cnc CENTER FOR NEUROSCIENCE AND CELL BIOLOGY UNIVERSITY OF COIMBRA PORTUGAL

cibb CENTRE FOR INNOVATIVE BIOMEDICINE AND BIOTECHNOLOGY

Índice

Welcome

Dear colleague,

After two challenging years of COVID-19, the Organizing Committee is pleased to announce that the 13th Symposium on the Biology of Acinetobacter will be held from June 21-23, 2023, in Coimbra, Portugal.

The meeting will focus on the recent developments of Acinetobacter spp. research and will bring experts from diverse scientific areas (clinical, antimicrobial resistance, environment, genetics, pathogenicity, new therapies, etc). The preliminary programme will be posted soon. We are also inviting you to share your work by submitting abstracts for selected oral communications and poster sessions.

The meeting will start on the Wednesday, June 21st, 2023 with a welcome reception in evening and on Thursday, June 22nd, we will have a dinner to get together in an informal environment. The meeting will finish in the evening of Friday, June 23rd. It is a unique opportunity to share novel scientific knowledge and to initiate collaborations in a friendly setting.

The conference will be held at The Auditorium of the Rectorate of the University of Coimbra, located at the Polo I of the University, Rua Larga 3000, close to the Old University. The University of Coimbra was established in 1290, one of the oldest universities in Europe, and in 2013 was added to the UNESCO World Heritage List in recognition of its historical buildings, open courtyard, cultural traditions and stunning city-wide views. Coimbra is located 50 km from the Atlantic Ocean. You can access the city from the international airports of Lisbon and Porto by train, bus or Coimbra shuttle. It is a touristic city and accommodation can be booked on this site, or independently if you wish, at your earliest convenience. Several options are provided on this site.

We all look forward to meeting soon again, and exchange inspiring discussions on the latest research findings in the Acinetobacter spp. field.

See you all in Coimbra!

Gabriela Jorge da Silva
Organizing Committee Chair

Committees

Local Organising Committee

Gabriela Jorge Da Silva (University of Coimbra, Portugal)

Cátia Caneiras (University of Lisbon, Portugal)

Maria José Saavedra (University of Trás-os-Montes e Alto Douro, Portugal)

Sara Domingues (University of Coimbra, Portugal)

Sérgio Mendes (University of Coimbra, Portugal)

Sofia Combo (University of Coimbra, Portugal)

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Laurent Poirel (University of Fribourg, Switzerland)

Luísa Peixe (University of Porto, Portugal)

Mario Feldman (Saint Louis, MO, USA)

Paul Higgins (University of Cologne, Germany)

Phil Rather (Emory University, Atlanta, GA, USA)

Sara Domingues (University of Coimbra, Portugal)

Programme

Wednesday – Day 21

02:00 pm | Opening Registration

04:00 pm | Welcome

Magnificent Dean of University of Coimbra, Prof. Doutor Amílcar Falcão

Dean of the Faculty of Pharmacy of the University of Coimbra, Prof. Doutor Fernando Ramos

Chair of Organizing Committee, Gabriela Jorge Da Silva (University of Coimbra, Portugal)

04:15 pm | OPENING LECTURE

"How Acinetobacter and I became close friends"

Harald Seifert (University of Cologne, Germany)

SESSION 1 | Acinetobacter spp.: Epidemiology and One Health (Ep 1H)

Chair: Paul Higgins (University of Cologne, Germany)

Co-chair: Ignasi Roca (University of Barcelona, Spain)

05:00 pm | Keynote Lecture 1

"Ecology of Acinetobacter baumannii – from nature to bedside"

Gottfried Wilharm (Robert Koch-Institute, Berlin, Germany)

05:30 pm | Oral Communications

O1-1
A. baumannii infection in animals: how zoonotic are they?
Amédée André, Julie Plantade, Pauline Durieu, Anne-Sophie Godeux, Céline Pouzot-Nevoret, Xavier Charpentier and, *Maria-Halima Laaberki.

VetAgro Sup, CIRI, CIRI, CIRI, VetAgro Sup, CIRI, VetAgro Sup-CIRI.

O1-2
Acinetobacter baumannii from turkeys reared for meat production and their environment
*A. Schmitz (1, 2), D. Hanke (2, 3), D. Lüschow (1, 2), S. Schwarz (2, 3), P. G. Higgins (4, 5, 6) and A. T. Feßler (2, 3)

(1) Institute of Poultry Diseases, School of Veterinary Medicine, Freie Universität Berlin, 14163 Berlin, Germany (2) Veterinary Centre for Resistance Research (TZR), Freie Universität Berlin, 14163 Berlin, Germany (3) Institute of Microbiology and Epizootics, Centre for Infection Medicine, School of Veterinary Medicine, Freie Universität Berlin, 14163 Berlin, Germany (4) Institute for Medical Microbiology, Immunology and Hygiene, Faculty of Medicine and University Hospital Cologne, University of Cologne, 50935 Cologne, Germany (5) German Center for Infection Research (DZIF), Partner Site Bonn-Cologne, 50935 Cologne, Germany (6) Center for Molecular Medicine Cologne, Faculty of Medicine and University Hospital Cologne, University of Cologne, 50935 Cologne, Germany.

O1-3
One Health and pan genomic epidemiology of a superbug

Valeria Mateo-Estrada (1), Ciara Tyrrell (2), Benjamin A. Evans (3), Fiona Walsh (2) and *Santiago Castillo-Ramírez (1)

1) Universidad Nacional Autónoma de México, Cuernavaca, México 2) Maynooth University, Maynooth, Ireland 3) University of East Anglia, Norwich, UK.

O1-4

High proportion and diversity of novel taxa among Acinetobacter isolates from cattle feces

*Martina Kyselková (1), Violetta Shestivska (2), Martina Maixnerová (2), Eva Skřivanová (3), Alexandr Nemec (2)

1 Laboratory of Environmental Microbiology, Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic; 2 Centre for Epidemiology and Microbiology, National Institute of Public Health, Prague, Czech Republic; 3 Department of Microbiology, Nutrition and Dietetics, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Prague, Czech Republic.

06:30 pm | Welcome Reception

Thursday – Day 22

SESSION 2 | Genetics and Evolution of Acinetobacter spp. (GE)

Chair: Beate Averhoff (Goethe- University Frankfurt/Main, Germany)

Co-chair: Sara Domingues (University of Coimbra, Portugal)

09:00 am | Keynote Lecture 2

"A multi-scale perspective of Acinetobacter evolution"

Ingo Ebersberger (Goethe – University Frankfurt/Main, Germany)

09:30 am | Oral Communications

O2-1
Genomic diversity of carbapenem-resistant Acinetobacter baumannii reveals distinct virulence evolution

*Mor Lurie-Weinberger1, Darya Bychenko1, Alona Keren-Paz1, David Swartz1 and Yehuda Carmeli1,2,3

1National Institute for Antibiotic Resistance and Infection Control, Israel Ministry of Health, Tel Aviv, Israel; 2Division of Epidemiology and Preventive Medicine, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel; 3Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.

O2-2
Structure and regulation of the hemO gene cluster for heme uptake in Acinetobacter baumannii

*D. Visaggio (1, 2, 3), I. Artuso (1), M. Lucidi (1, 2), P. Visca (1, 2, 3)

1 Department of Science, Roma Tre University, Rome, Italy; 2 NBFC, National Biodiversity Future Center, Palermo, Italy; 3 Santa Lucia Foundation IRCCS, Rome, Italy.

O2-3
Genetic manipulation of A. baumannii-infecting bacteriophages to tackle a larger range of capsular types

*Rita Domingues 1,2; Hugo Oliveira 1,2; Joana Azeredo 1,2

1CEB-Centre of Biological Engineering, University of Minho, Braga, Portugal 2LABBELS –Associate Laboratory, Braga/Guimarães, Portugal.

O2-4
Induction and evolutionary conservation of natural transformation in Acinetobacter baumannii

Jason Chirakadavil (1), Ludovic Poiré (1), Fanny Mazzamurro (2), Eduardo Rocha (2), Maria-Halima Laaberki (1), *Xavier Charpentier (1)

1 Centre Internation de Recherche en Infectiologie. Lyon. France; 2 Institut Pasteur, Paris, France.

10:30 am | Coffee Break

SESSION 3 | Bacterial Infections (Inf)

Chair: Mario Feldman (Washington University St Louis, USA)

Co-Chair: Alejandra Mussi (National University of Rosario, Argentina)

11:00 am | Keynote Lecture 3

"New players in biofilm lifestyle in Acinetobacter baumannii"

Emmanuelle Dé (University of Rouen, France)

11:30 am | Oral Communications

O3-1
Dietary zinc deficiency compromises immunity to Acinetobacter baumannii pneumonia

Dziedzom A. Bansah (1), Xiaomei Ren (1), Zachery R. Lonergan (2), Lillian J. Juttukonda (2), Christopher Pinelli (2), Kelli L. Boyd (2), Eric P. Skaar (2), *Lauren D. Palmer (1)

1 University of Illinois Chicago, Chicago, IL USA 2 Vanderbilt University Medical Center, Nashville, TN, USA.

O3-2
Elucidating the role of Acinetobacter calcoaceticus in promoting intestinal inflammation

*Janiece Glover, Brittney D. Browning (3), Taylor D. Ticer (2), Sarah A. Dooley (1), Jessica Digrazia (1), Amy C. Engevik (1), Melinda A. Engevik (1,2)

Regenerative Medicine and Cell Biology (1), Microbiology and Immunology (2), Institute of Psychiatry (3), Medical University of South Carolina, Charleston, SC United States.

O3-3
Epithelial-macrophage communication promotes clearance of Acinetobacter baumannii from the airway

Jisun Kim, Gyu-Lee Kim, *Dane Parker

Department of Pathology, Immunology and Laboratory Medicine, Center for Immunity and Inflammation, Rutgers New Jersey Medical School, Newark, New Jersey, USA.

O3-4
Mechanistic insights into the phagocytosis resistance of the WHO priority pathogen Acinetobacter baumannii

*Juliette Van Buylaere (1, 2), Etienne Robino (1, 2), Clémence Whiteway (1, 2) and Charles Van der Henst (1, 2)

1 Microbial Resistance and Drug Discovery/VIB-VUB Center for Structural Biology, Brussels, Belgium; 2 Structural Biology Brussels/Vrije Universiteit Brussel, Brussels, Belgium.

12:30 pm | Lunch

01:30 pm | Poster Session (Topics 1 – 4):

P01
ID22 – Characterization of 85 Acinetobacter baumannii strains by sequence typing, antibiotic resistance, CRISPR-Cas systems, and phage sensitivity | **Martina Scarrone**

P02
ID39 – Epidemiology and heterogeneity of genotypes and phenotypes of the A. baumannii strains | **Adam Valcek**

P03
ID52 – MALDI-TOF MS-based approach to uncover new Acinetobacter taxa in cattle feces | **Violetta Shestivska**

P04
ID54 – Characterization of tigecycline (tet(X3)) and multidrug-resistant Acinetobacter lwoffii/pseudolwoffii from French animals | **Agnese Lupo**

P05
ID55 – Proposal for Acinetobacter higginsii sp. nov. to accommodate organisms of human clinical origin previously classified as Acinetobacter genomic species 16 | **Alexandr Nemec**

P06
ID56 – Acinetobacter bovis sp. nov., a small-chromosome species found in cattle feces | **Alexandr Nemec**

P07
ID59 – Species distribution and antibiotic susceptibility of Acinetobacter isolates from non-hospitalized patients | **Alexandr Nemec**

P08
ID60 – The classification of Acinetobacter species in the light of phylogenomics | **Alexandr Nemec**

P09
ID101 – Frequency of Acinetobacter species isolated from clinical samples over a 34-month period | **Paul G Higgins**

P10
ID133 – Characterization of NDM-producing Acinetobacter bereziniae strains isolated in Chilean hospitals | **Paul G Higgins**

P11
ID154 – Phenotypic and genotypic characterization of Acinetobacter baumannii clinical isolates: yesterday and today | **Astri D. Tagueha**

P12
ID157 – Acinetobacter baumannii in a primary hospital from Portugal: distribution and antibiotic susceptibility patterns during five years (2018-2023) | **Adriana Pedrosa**

P13
ID92 – Isolation and characterisation by whole genome sequencing of Acinetobacter spp. collected from raw meat and meat products in Spain | **Alba Puente**

P14
ID71 – Acinetobacter abundance in cattle feces from Czech farms | **Anitha Ravi**

P15
ID46 – Hi-GRIL-seq uncovers a posttranscriptional regulator of CarO and BfnH in Acinetobacter baumannii AB5075 | **Fergal J. Hamrock**

P16
ID74 – Transcriptomic analysis of A. baumannii AB5075 with (differential)-RNA sequencing | **Maha M. Sulimani**

P17
ID78 – Characterization of a genome database of 837 isolates assigned to 72 distinct species in the Acinetobacter genus using the Pasteur Multi-locus sequencing type (MLST) scheme. | **Antonella Migliaccio**

P18
ID96 – Identification of the minimal region involved in the replication of a widespread Acinetobacter plasmid family carrying an NDM-1 gene | **Miguel Angel Cevallos**

P19
ID141 – Acinetobacter baumannii bacteraemia: clinical aspects and molecular characterization | **Mohamed Azzedine Bachtarzi**

P20
ID129 – Ancestral state reconstruction of multi-drug resistant Acinetobacter species. | **Georgi Merhi**

P21
ID164 – Transposition of ISAba13 in AB5075 | **Charles Cooper**

P22
ID165 – Genomic epidemiology and evolution of globally distributed carbapenem resistant Acinetobacter baumannii | **Evangelos Mourkas**

P23
ID167 – The Acinetobacter baumannii genome and sequence reference libraries hosted on PubMLST.org | **Samuel Sheppard**

P24
ID50 – Role of the competence protein ComC of Acinetobacter baumannii in natural transformation, twitching motility and adhesion | **Katharina Pfefferle**

P25
ID44 – The VBNC state as global stress response in different Acinetobacter baumannii strains | **Patricia König**

P26
ID15 – Within-host microevolution of multidrug resistant Acinetobacter baumannii | **Léa Bednarczuk**

P27
ID168 – Acinetobacter baumannii outer-membrane vesicles immunomodulate human pulmonary epithelial cells: effects of antimicrobial resistance | **Sofia Combo**

P28
ID26 – First molecular characterisation of colistin and carbapenem-resistant clinical isolate of Acinetobacter baumannii from Bosnia and Herzegovina | **Sanja Jakovac**

P29
ID43 – Misidentification among Acinetobacter spp. species other than A.baumannii with analysis of their resistance profiles and acquired resistance genes. | **Tomasz Kasperski**

P30
ID145 – Clonal Outbreak of Multidrug-resistant Acinetobacter baumannii isolates harbouring the blaOXA-24/40 carbapenemase gene in an Intensive Care Unit in a Tertiary Hospital in Northern Spain | **Maitane Aranzamendi**

P31
ID61 – Characterization of the subclass B3 Metallo-ß-lactamase ANB-1 from Acinetobacter nosocomialis | **Laurent Poirel**

P32
ID63 – Characterisation of Chloramphenicol Resistance Genes in Acinetobacter baumannii | **Orlaith Plunkett**

P33
ID66 – Evaluation of novel immunological rapid test (Resist Acineto) Rapid Detection of acquired Carbapenemase Producers in Acinetobacter sp | **Maxime Bouvier**

P34
ID67 – Dissemination of pan-aminoglycoside resistance gene armA and carbapenemase gene blaOXA-23 Acinetobacter baumannii Global Clone 2 in Switzerland, 2020-2021 | **Maxime Bouvier**

P35
ID106 – Insights into the genetic contexts of sulfonamide resistance among early clinical isolates of Acinetobacter baumannii | **Nabil Karah**

P36
ID120 – Fast and efficient method for detection of Carbapenem Resistant Acinetobacter baumannii from screening samples | **Alona Keren-Paz**

P37
ID122 – A new MFS efflux pump and its putative regulator are involved in the antimicrobial resistance, virulence and surface-associated motility of Acinetobacter baumannii | **Marc Gaona**

P38
ID124 – Surveillance, control and characterization of an NDM-1 Acinetobacter baumannii outbreak | **Kyriaki Xanthopoulou**

P39
ID125 – Development of an immunochromatographic lateral flow assay to rapidly detect OXA-23-, OXA-40-, OXA-58- and NDM-mediated carbapenem resistance determinants in Acinetobacter baumannii | **Alexander Klimka**

P40
ID138 – Evolutionary drift of Carbapenem Resistant Acinetobacter baumannii in the Israel | **Darya Bychenko-Banyas**

P41
ID139 – Acquisition of ciprofloxacin resistance is associated with increased biofilm formation and altered motility in acinetobacter baumannii | **Sérgio G. Mendes**

P42
ID140 – Polyclonal NDM producers Acinetobacter sp on Algerian hospital | **Mohamed Azzedine Bachtarzi**

P43
ID143 – Carbapenemase production in no fermentative Gram negative bacilli in Algeria | **Mohamed Azzedine Bachtarzi**

P44
ID144 – Acintobacter baumannii: first etiology of nosocomial meningitis in the era of covid-19 at the university hospital of algiers | **Mohamed Azzedine Bachtarzi**

P45
ID142 – Acinetobacter baumannii: respiratory infections versus other clinical forms | **Mohamed Azzedine Bachtarzi**

P46
ID155 – Unraveling the Genetic Mechanisms of blaIMP-5 Dissemination in Acinetobacter Species: Insights and Unanswered Questions | **Filipa Grosso**

P47
ID163 – Heteroresistance to cefiderocol in Acinetobacter baumannii is not a cause of the imbalance in mortality observed in CREDIBLE-CR | **Christopher Longshaw**

P48
ID109 – Emergence of multidrug-resistant Acinetobacter baumannii isolates carrying blaGES-35/blaOXA-23 and blaGES-11/blaOXA-23 genes in Alexandria, Egypt | **Ainhoa Molins-Bengoetxea**

P49
ID5 – Genomic analysis of tigecycline-resistant and extensively drug-resistant Acinetobacter baumannii

harbouring a conjugative plasmid containing aminoglycoside resistance transposon TnaphA6 | **Satoshi Nishida**

03:00 pm | ***Round Table: Heterogeneity in Acinetobacter baumannii: pitfall or useful resource?***

Chair: Charles Van Der Henst (University of Vrije, Belgium)

04:00 pm | ***The Acinetobacter baumannii website (Ab-web): A multidisciplinary knowledge hub, communication platform, and workspace***

Nabil Karah et al. (Umeå University, 901 87 Umeå, Sweden)

04:10 pm | Coffee Break

SESSION 4 | Antibiotic Resistance Mechanisms (AMR)

Chair: Laurent Poirel (University of Fribourg, Switzerland)

Co-Chair: Nabil Karah (University of Umea, Sweden)

04:30 pm | Keynote Lecture 4

"Multidrug-resistant Acinetobacter baumannii: main resistance mechanisms and novel therapeutic alternatives"

Laurent Poirel (University of Fribourg, Switzerland)

05:00 pm | Oral Communications

O4-1
Phage-mediated transmission of colistin resistance in Acinetobacter baumanni

*M. Lucidi (1, 2), G. Capecchi (2), I. Artuso (2), S. Traditi (2), D. Visaggio (2, 3), F. Imperi (2, 3), P. Visca (2, 3).

1 NBFC, National Biodiversity Future Center, piazza Marina 61, 90133 Palermo, Italy; 2 Department of Science, Roma Tre University, Viale G. Marconi 446, 00146 Rome, Italy; 3 Santa Lucia Foundation IRCCS, Via Ardeatina 306/354, 00179 Rome, Italy

O4-2
Resistome and Virulome of cefiderocol resistant Acinetobacter baumannii clinical isolates from Alexandria, Egypt

*Sandra Sanchez-Urtaza1, Ainhoa Molins-Bengoetxea1, Alain Ocampo-Sosa2,3, Marta Hernández4, David Abad4, Mohammed A. El-Kholy5, Sherine M. Shawky6, Itziar Alkorta7, Lucia Gallego1

1Laboratory of Antibiotics and Molecular Bacteriology, Faculty of Medicine and Nursing, University of the Basque Country, Leioa, Spain 2Microbiology Department of Hospital Marqués de Valdecilla-IDIVAL, Santander, Spain 3CIBER de Enfermedades Infecciosas (CIBERINFEC), Instituto de Salud Carlos III, Madrid, Spain 4Laboratory of Molecular Biology and Microbiology, One health, Agrarian Technological Institute of Castile and Leon (ITACyL), Valladolid, Spain. 5Department of Microbiology and Biotechnology, Division of Clinical and Biological Sciences, College of Pharmacy, Arab Academy for Science, Technology & Maritime Transport (AASTMT), Alexandria, Egypt 6Medical Research Institute, Alexandria University, Alexandria, Egypt 7Department of Biochemistry and Molecular Biology, Faculty of Science and Technology, University of the Basque Country, Leioa, Spain

O4-3
Transfer of the chromosomally encoded OXA-23 carbapenemase by recombination in Acinetobacter baumannii

*A. Kyriaki Xanthopoulou (1,2), B. Mónica Cerezales (3), C. Alexander T Dilthey (4), D. Julia Wille (1,2), E. Kai Lucaßen (1), F. Harald Seifert (1,2), G. Lucía Gallego (3), H. Paul G Higgins (1,2)

1 Institute for Medical Microbiology, Immunology and Hygiene, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany 2 German Center for Infection Research, Partner site Bonn-Cologne, Cologne, Germany 3 Acinetobacter baumannii Research Group. Faculty of Medicine and Nursing, Department of Immunology, Microbiology, and Parasitology, University of the Basque Country UPV/EHU, Leioa, Spain 4 Institute of Medical Microbiology and Hospital Hygiene, Heinrich Heine University Düsseldorf, Düsseldorf, Germany

O4-4

Increased Intraspecies Transfer of blaCTX-M-1 After Interspecies Acquisition from Salmonella enterica to Acinetobacter baumannii by Natural Transformation

*Tiago Lima (1,2), Gabriela Jorge da Silva (1,2), Sara Domingues (1,2).

1 Faculty of Pharmacy of University of Coimbra, Coimbra, Portugal; 2 Center for Neurosciences and Cell Biology of University of Coimbra, Coimbra, Portugal

07:00 pm | Conference dinner

Friday – Day 23

SESSION 5 | Acinetobacter spp. Pathogenicity (P)

Chair: Phil Rather (Emory University, Atlanta, USA)

Co-chair: Lauren Palmer (University of Illinois, Chicago, USA)

09:00 am | Keynote Lecture 5

"Dissecting the virulence strategies of Acinetobacter baumannii"

Mario Feldman (Washington University St Louis, USA)

09:30 am | Oral Communications

O5-1
Structural basis for Acinetobacter baumannii biofilm formation

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

Joint Biotechnology Laboratory, MediCity, Faculty of Medicine, University of Turku, Turku, Finland.

O5-2
Epinephrine exposure facilities the adaptation of Acinetobacter baumannii to hostile host environments and concomitantly promotes the polymicrobial interactions

*H. Le (1), J. Hardouin (1,2), V. Perrot (1), T. Jouenne (1), P. Cosette (1,2), E. Dé (1)

(1) Normandie Univ, UNIROUEN, INSA Rouen, CNRS, Polymers, Biopolymers, Surfaces Laboratory, Rouen, France. (2) PISSARO Proteomic Facility, IRIB, Mont-Saint-Aignan, France.

O5-3
Intracellular Acinetobacter baumannii in vivo: potential roles in UTI and respiratory infections

*Gisela Di Venanzio (1), Jesus S Distel (1), Jennie E Hazen (1,2), Joseph J Mackel (3), David A Rosen (3), Scott J Hultgren (1,2), Mario F Feldman (1)

1 Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO 63110, USA. 2 Department of Molecular Microbiology, Center for Women's Infectious Disease Research, Washington University School of Medicine, St. Louis, MO 63110, USA. 3 Department of Pediatrics, Division of Infectious Diseases, Washington University School of Medicine, Saint Louis, MO, United States.

O5-4
Desiccation induces apparent death in the pathogenic bacterium Acinetobacter baumannii

*G. Capecchi (1), M. Lucidi (1, 2), D. Visaggio (1, 2, 3), I. Artuso (1), C. Spagnoli (1), L. Persichetti (1), E. Fardelli (1), G. Capellini (1), G. Rampioni (1, 3), L. Leoni (1), F. Imperi (1, 2, 3), P. Visca (1, 3).

1 Department of Science, Roma Tre University, Rome, Italy; 2 NBFC, National Biodiversity Future Center, Palermo, Italy; 3 Santa Lucia Foundation IRCCS, Rome, Italy.

10:30 am | Coffee Break

SESSION 6 | Prevention and Treatment of Acinetobacter spp. Infections (PT)

Chair: Harald Seifert (University of Cologne, Germany)

Co-chair: Hugo Oliveira (University of Minho, Portugal)

11:00 am | Keynote Lecture 6

"Utilising bacteriophages to combat multidrug-resistant Acinetobacter baumannii infections"

Jeremy Barr (Monash University, Melbourne, Australia)

11:30 am | Oral Communications

O6-1
Targeting multidrug-resistant Acinetobacter baumannii to develop next-generation antibiotics

*Anke Breine (1, 2), Els Pardon (2), Jan Steyaert (2), Han Remaut (2) and Charles Van der Henst (1, 2)

1 Microbial Resistance and Drug Discovery/VIB-VUB Center for Structural Biology, Brussels, Belgium; 2 Structural Biology Brussels/Vrije Universiteit Brussel, Brussels, Belgium.

O6-2
OXA-23 β-lactamase Overexpression in Acinetobacter baumannii results in cellular vulnerabilities that can be targeted for new drug development

*Jennifer M Colquhoun, Philip N Rather

Department of Microbiology and Immunology, Emory University, Atlanta, Georgia, USA.; Emory Antibiotic Resistance Center, Emory University, Atlanta, Georgia, USA; Research Service, Atlanta VA Medical Center, Decatur, Georgia, USA.

O6-3
Bacteriophage susceptibility in Acinetobacter baumannii – Capsule type as prerequisite to predict phage lysis?

*A. Annika Yanina Classen (1,2,3), B. Christine Rohde (4), C. Clara Rolland (4), D. Maria J. G. T. Vehreschild (3, 5), E. Johannes Wittmann (4), F. Kyriaki Xanthopoulou (1,3), G. Harald Seifert (1,3), H. Paul Higgins (1,3)

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O6-4
To Combat Drug-resistant Bacteria from a Chemist's Point of View

*Anren Hu (1), K. Chang (1,2)

1 Department of Laboratory of Medicine and Biotechnology, College of Medicine, Tzu-Chi University, Hualien, Taiwan; 2 Department of Laboratory Medicine, Buddhist Tzu-Chi General Hospital, Hualien, Taiwan

12:30 pm | Lunch

01:30 pm | Poster Session (Topics 4 – 7)

P51
ID147 – Differential activity of Rho and CsrA in subpopulations of Acinetobacter baumannii regulate a switch between virulent and avirulent states. | **Philip Rather**

P52
ID160 – Csu pili dependent biofilm formation and virulence of Acinetobacter baumannii | **Irfan Ahmad**

P53
ID6 – Genomics of Acinetobacter baumannii iron uptake | **Irene Artuso**

P54
ID49 – The capsule of Acinetobacter baumannii: roles and regulation | **Clemence Whiteway**

P55
ID30 – Cryo-electron Microscopy Structure of the Zifanocycline-Bound Ribosome from Acinetobacter baumannii Reveals a New Potential Binding Site of Ribosome | **Xiaoting Hua**

P56
ID37 – Efficacy of melittin combined with antibiotics against carbapenemase-producing Acinetobacter baumannii clinical strains | **Tania Cebrero-Cangueiro**

P57
ID38 – Efficacy of N-desmethyltamoxifen alone and in combination with colistimethate sodium and tigecycline in experimental pneumonia model caused by Acinetobacter baumannii clinical strains. | **Soraya Herrera-Espejo**

P58
ID41 – Development of an Immunoinformatic Based Multi-Epitope Vaccine Against Acinetobacter baumannii | **Sean Jeffreys**

P59
ID51 – Targeting iron homeostasis as a means to potentiate colistin treatment in MDR Acinetobacter baumannii | **Kavita Gadar**

P60
ID69 – The artificial sweetener acesulfame-K inhibits growth of multidrug resistant Acinetobacter baumannii and potentiates carbapenem activity | **Rubén de Dios**

P61
ID95 – Genome analysis of Acinetobacter strains with antifungal properties isolated from amphibians and from the nosocomial setting | **Miguel Angel Cevallos**

P62
ID97 – Antimicrobial, antibiofilm and antivirulence activity of glucocorticoid PYED-1 against Acinetobacter baumannii | **Maria Stabile**

P63
ID104 – Bactericidal Efficacy Analysis of Silver Nanoparticles Synthesized from Bitter Gourd Extract | **Jia-Yu Hu**

P64
ID162 – Bacteriocins as promising new weapon against the nosocomial pathogen Acinetobacter baumannii | **Tristan Rubio**

P65
ID113 – Acinetobacter baumannii OmpA-like porins: functional characterization in bacterial physiology, antibiotic-resistance, and virulence | **Cecilia Ambrosi**

P66
ID29 – Discovery of BfmR inhibitor in combination with Meropenem with Potent activity against Carbapenem resistance Acinetobacter baumannii | **Xiaoting Hua**

P67
ID3 – Acinetobacter baumannii is able to survive in natural soil for over four years | **Jasna Hrenovic**

P68
ID34 – Evaluation of deaD as a component of the persistence molecular mechanism of Acinetobacter baumannii | **Sílvia Dias de Oliveira**

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ID57 – Assembling the Acinetobacter baumannii surface: Exploring novel aspects of lipooligosaccharide synthesis | **Leah VanOtterloo**

P70
ID64 – Initial characterisation of the twin-arginine translocation system in Acinetobacter baumannii AB5075 | **Rebecca Nolan**

P71
ID68 – An AT3 family acyltransferase participates in Acinetobacter baumannii nutrient metal acquisition and virulence | **Dillon E. Kunkle**

P72
ID77 – Transcriptional profiling of Acinetobacter baumannii during antibiotic and environmental stress | **Ali Bakheet**

P73
ID114 – The protein HslJ boosts Acinetobacter baumannii survival against oxidative stress | **Daniela Scribano**

P74
ID126 – Differential expression of the AdeABC RND efflux pump and its regulator during motility in Acinetobacter baumannii | **Rocío Arazo del Pino**

P75
ID128 – Lights modulates resistance to desiccation in A. baumannii | **María Alejandra Mussi**

P76
ID131 – Contribution of Fourier Transform Mass Spectrometry to the study of the lipidome of Acinetobacter baumannii | **D. Vergoz**

P77
ID146 – Domain-architecture aware phylogenetic profiling indicates a functional diversification of type IVa pili in Acinetobacter baumannii | **Ruben Iruegas**

P78
ID161 – Alleles selected by growth in long-term stationary phase | **Phoebe Lostroh**

P79
ID20 – Repressor of the SOS Response Mechanism in Acinetobacter baumannii requires Helix-Formation and Dimerization for its DNA-binding Ability | **Belinda Candra**

SESSION 7 | Physiology and Metabolism (FisMet)

Chair: Ingo Ebersberger (Goethe- University Frankfurt/Main, Germany)

Co-chair: Cátia Caneiras (University of Lisbon, Portugal)

02:30 pm | Keynote Lecture 7

"The response of Acinetobacter baumannii to stress during the pathogenesis of pneumonia"

Eric Skaar (Vanderbilt University, Nashville, TN, USA)

03:00 pm | Oral Communications

O7-1
Light Signal Transduction and Chronobiology in the Human Pathogen Acinetobacter baumannii: Role of the BfmRS two component system

*B. Perez Mora1; Permingeat, Valentín1; *Mussi, María Alejandra1

1- Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI), CONICET, Rosario, Argentina.

O7-2
Molecular interactions between peptidoglycan integrity maintenance and outer membrane lipid asymmetry in Acinetobacter baumannii

Sinjini Nandy, Misha I. Kazi, *Joseph M. Boll

University of Texas at Arlington, Arlington, US

O7-3
Systematic Dissection of Genetic Vulnerabilities in Acinetobacter baumannii

*R. Ward (1, 2), J. Tran (1, 3), A. Banta (1, 4), E. Bacon (1, 3), W. Rose (5), and J. Peters (1, 4, 6, 7, 8)

1 School of Pharmacy, University of Wisconsin-Madison, USA; 2 Laboratory of Genetics, University of Wisconsin-Madison, USA; 3 Microbiology Doctoral Training Program, University of Wisconsin-Madison, USA; 4 Great Lakes Bioenergy Research Center, University of Wisconsin-Madison, USA; 5 Pharmacy Practice Division, University of Wisconsin-Madison, USA; 6 Department of Bacteriology, University of Wisconsin-Madison, USA; 7 Department of Medical Microbiology and Immunology, University of Wisconsin-Madison, USA; 8 Center for Genomic Science Innovation, University of Wisconsin-Madison, USA.

O7-4
Identification and characterization of a novel pathway for aldopentose degradation in Acinetobacter baumannii ATCC 19606

*Lydia Alberti, Patricia König, Sabine Zeidler, Volker Müller and Beate Averhoff

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

04:00 pm | CLOSING LECTURE

” Adaptation and persistence of Acinetobacter baumannii: metabolism and the VBNC state “

Beate Averhoff (Goethe-University Frankfurt/Main, Germany)

04:30 pm | Farewell

BioCyc Genome Database Collection

Microbial Genomes Web Portal

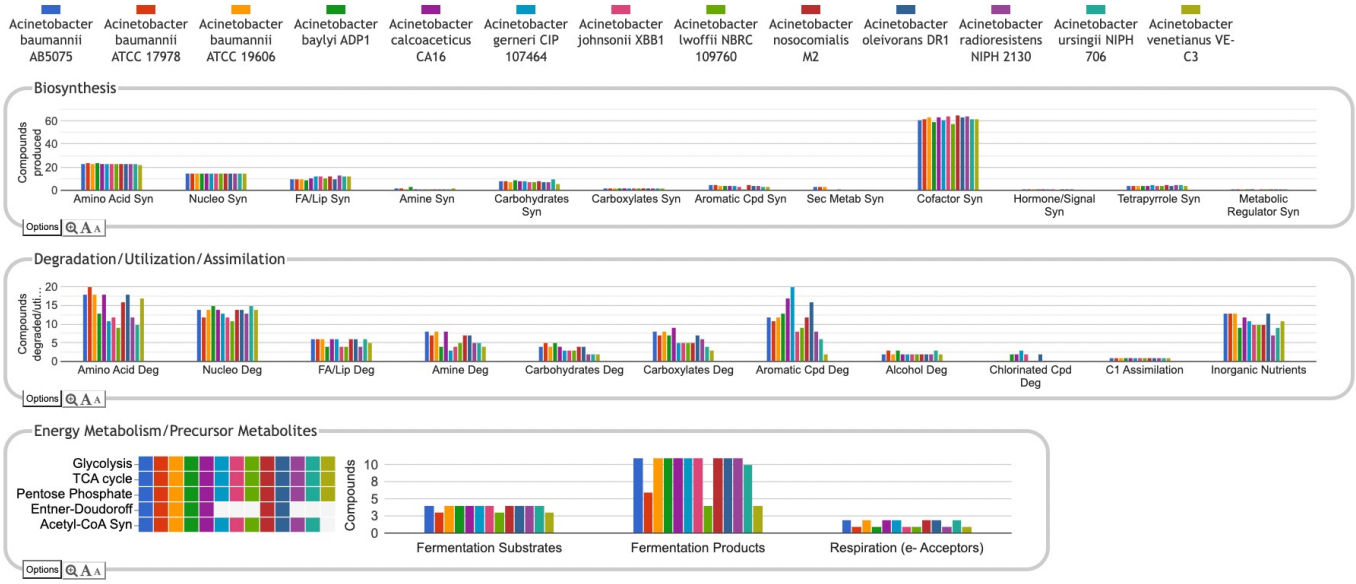
Free BioCyc Tutorial Highlighting Acinetobacteria Databases and Bioinformatics Tools

Mon, June 26, 2023, 5pm London/9am San Fran time

Register at https://tinyurl.com/yv38vwjz

BioCyc Comparative Genome Dashboard Tool

The Comparative Genome Dashboard enables visual comparisons of the overall biological capabilities of one or more organisms from their genome and pathway annotations. Below is a comparison of 13 Acinetobacter strains. The top two panels show the compounds they produce and degrade; the bottom panel shows the energy metabolism processes they use, the compounds used or produced for fermentation, and the compounds used as electron acceptors.



Acinetobacter Pathway/Genome DataBases (PGDBs)

- 295 *Acinetobacter* databases encompassing 53 species
- 2 manually curated databases:
  - *A. baumannii* ATCC 17978 and AB5075
  - Information from > 80 publications
  - Experimental evidence for more than 200 genes, 40 pathways and 100 complexes
- Biolog Phenotype Microarray datasets for 4 PGDBs including strain ATCC 17978
- 68 small RNAs curated for strain AB5075

Extensive Bioinformatics Tools

- Genome browser, BLAST search, sequence pattern search
- Pathway diagrams, metabolic network browser, metabolic route search
- Powerful omics data analysis tools:
  - Paint metabolite data onto pathway diagrams and full metabolic networks
  - Enrichment analysis
  - Pathway activation calculator
  - Omics Data Table Viewer
- Comparative genome and pathway analyses

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Abstracts

Oral Comunications

The Acinetobacter baumannii website (Ab-web): A multidisciplinary knowledge hub, communication platform, and workspace

Nabil Karah

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Nabil Karah (1,2), Valeria Mateo-Estrada (3), Santiago Castillo-Ramírez (3), Paul G Higgins (4,5), Benjamin Havenga (6), Wesaal Khan (6), Sara Domingues (7,8), Gabriela Jorge Da Silva (7,8), Laurent Poirel (9,10), Patrice Nordmann (9,10), Cecilia Ambrosi (11), Chaoying Ma (12), Siobhán McClean (12), María Paula Quiroga (13), Verónica E Alvarez (13), Daniela Centron (13), Raffaele Zarrilli (14), Johanna J Kenyon (15), Thomas A Russo (16), Benjamin A Evans (17), Andres Opazo-Capurro (18), Rayane Rafei (19), Monzer Hamze (19), Ziad Daoud (20,21), Irfan Ahmad (1,22), Philip N Rather (23,24), Ruth M Hall (25), Gottfried Wilharm (26), Bernt Eric Uhlin (1)

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Acinetobacter baumannii is a Gram-negative bacterium increasingly implicated in hospital-acquired infections and outbreaks. Effective prevention and control of such infections are commonly challenged by the frequent emergence of multidrug-resistant strains. Here we introduce Ab-web (https://www.acinetobacterbaumannii.no), the first online platform for sharing expertise on A. baumannii. Ab-web is a species-centric knowledge hub, initially with ten articles organized into two main sections, “Overview” and “Topics”, and three themes, “epidemiology”, “antibiotic resistance”, and “virulence”. The “workspace” section provides a spot for colleagues to collaborate, build, and manage joint projects. Ab-web is a community-driven initiative amenable to constructive feedback and new ideas.

Session 1 - Epidemiology and One Health (Ep 1H)

O1-1 A. baumannii infection in animals: how zoonotic are they?

Maria-Halima Laaberki

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Amédée André, Julie Plantade, Pauline Durieu, Anne-Sophie Godeux, Céline PouzotNevoret, Xavier Charpentier and, Maria-Halima Laaberki.

VetAgro Sup, CIRI, CIRI, CIRI, VetAgro Sup, CIRI, VetAgro Sup-CIRI

Background: Domestic animals can develop serious infections caused by Acinetobacter species, including by A. baumannii (AB). Several studies report infections in pets that are primarily nosocomial but also community-acquired. Strain sequence types associated with pet diseases (ST2, ST25) are also the same one found associated with disease in humans. However, typing alone may be insufficient to clearly understand the epidemiological link between human and animal infections. Methods: To better identify the origin of nosocomial infections in pets, we studied the carriage of A. baumannii in veterinary hospital services between year 2021 and 2022 with 94 pets (cats, dogs) screened for AB oral carriage (cf. Figure). Strain isolated were subjected to hybrid genome sequencing (ONT and Illumina). Nanopore sequencing reads were basecalled using the 'super accuracy' model from Guppy. Genome were assembled using Unicycler, annotated using Prokka. Closest genomes were identified using the WhatsGNU tool.

Results: Over the course of a year, AB carriage was studied in pets hospitalized in an emergency unit (ICU). About 14% of the animals were carriers of AB with comparable contamination between dogs and cats. Duration of hospitalization was identified as a risk factor for contamination ($p<0.05$, Fisher's exact test). The latter result suggested potential contamination from the ICU environment that was therefore. Whole genome sequencing of the 13 strains showed that most of them belonged to the ST2(IP) type and, among them, nine to the ST350(Oxford) type. Genomic analysis and antibiotic susceptibility testing demonstrated the clonal spread of a multi-antibiotic resistant strain. In depth core and accessory genome comparison (resistance island, mobile genetic elements) with publicly available genome further support proximity to human-associated isolates.

Conclusion: Although AB can cause infections in humans and various animal species, the scientific literature reports few descriptions of true zoonotic transmissions with clones presumably transmitted from human to animal. Our study provides evidence of potential anthropogenic contamination, human to animal transmission and persistence of a strain in animal care facilities.

O1-2 Acinetobacter baumannii from turkeys reared for meat production and their environment

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Background: There are few data on A. baumannii isolated from farm animal populations and their environment. Concerning poultry, an outbreak in a commercial chicken farm in China, as well as occasional findings in chicken and geese have been described. The detection of isolates from raw turkey and chicken meat might, therefore, potentially pose a threat to public health.

Methods: In this study, a total of 643 samples from turkeys, including 250 environmental and 393 diagnostic samples, were examined for the presence of A. baumannii. Species were identified via MALDI-TOF-MS. Pulsed-field gel electrophoresis was conducted for preliminary characterisation. Antimicrobial and biocide susceptibility were tested by broth microdilution, and 26 isolates were further examined using whole-genome sequencing (WGS).

Results: From a total of 643 samples, 99 A. baumannii isolates were obtained (Table 1). The majority of isolates (n=96) with a detection rate of 79.7% originated from chick-box-papers (meconium samples) of one-day-old turkey chicks. Two isolates came from boot swabs before slaughter, and one isolate was cultured from a lung-heart swab. The distributions of the minimal inhibitory concentration values were unimodal for the biocides tested. The highest resistance rates were detected for ciprofloxacin (17%) and no multidrug resistance properties were detected. WGS revealed 16 Pasteur and 18 Oxford sequence types (ST). ST25, which is associated with the international clone 7, was the most common (n=9). Core genome MLST highlighted the diversity of most isolates.

Conclusion: We conclude that the presence of A. baumannii in samples from poultry can vary considerably, is transient, and was confined almost exclusively to chick-box-papers from one-day old chicks. The isolates are highly diverse and still susceptible to many antimicrobial agents. There is no evidence for a preference of A. baumannii for avian hosts.

O1-3 One Health and pan genomic epidemiology of a superbug

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1) Universidad Nacional Autónoma de México, Cuernavaca, México 2) Maynooth University, Maynooth, Ireland 3) University of East Anglia, Norwich, UK

Background: Antibiotic resistance is a major threat to human and animal health. In this regard, antibiotic-resistant Acinetobacter baumannii is one of the most important nosocomial pathogens nowadays. However, we know very little about this bacterial species outside of the clinic. Here, we aim to determine the evolutionary relationships (and antibiotic resistance potential) of isolates sampled from grass and animals to the major human International Clones.

Methods: Bacterial isolation, genome sequencing, and antimicrobial susceptibility testing of animal and grass isolates. Phylogenomic inference of animal and grass isolates plus publicly available genomes from the main human clones.

Results: Our core and accessory genomic epidemiology analyses showed that animal and grass isolates cluster in clear clades well differentiated from one another and from the major human clones. Furthermore, the use of the accessory genome was extremely useful to resolve clades with little resolution. Additionally, both the animal and grass clades were not resistant to clinically relevant antimicrobials. Conclusions: Our analyses demonstrate that animal and grass clones are distantly related to the major human clones and appear to have limited antibiotic resistance potential. Given our results, we suggest that surveillance of this species must go beyond the clinical settings and consider the environment in a clear One Health approach.

O1-4 High proportion and diversity of novel taxa among Acinetobacter isolates from cattle feces

Martina Kyselková

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Background: Livestock manure is an important soil fertilizer and its bacterial composition deserved attention. However, previous studies mainly focused on putative human pathogens such as A. baumannii, while little attention was paid to other Acinetobacter spp. In the frame of a project assessing the impact of antibiotic use in Czech cattle farms on the occurrence and antibiotic resistance of Acinetobacter spp., we present analysis of Acinetobacter isolates from cattle feces collected in 28 farms with contrasting antibiotic use.

Methods: One composite fecal sample from the farm floor was analyzed per farm. Acinetobacter isolates were obtained after sample enrichment in liquid mineral medium supplemented with sodium acetate as a sole carbon source at 30°C and subsequent plating onto both mineral agar with sodium acetate and CHROMagar™. Purified and dereplicated isolates were identified by MALDITOF MS using the current Bruker database supplemented with the homemade entries of additional Acinetobacter taxa. In addition, the rpoB gene was partially sequenced, as an independent taxonomic marker. The species identification was based on a combination of MALDI-TOF MS and rpoB sequencing. Antibiotic resistance of isolates was determined with disc diffusion test. Nonmetric multidimensional scaling and PERMANOVA were used to assess the relationship between in-farm antibiotic use and the frequency of occurrence of Acinetobacter species.

Results: In total, 245 Acinetobacter isolates were obtained from the 28 farms, with 58% isolates identified to 14 validly named species. The remaining 42% isolates either were allocated to 14 taxa representing putative novel species (up to 21 isolates per taxon) or represented taxonomically unique singletons. A. indicus, A. pseudolwoffii, and A. gandensis were the most common species and occurred in the highest numbers of farms (20, 20, and 13, respectively). In contrast, only three A. baumannii from two farms were obtained, all being wild-type susceptible, without having any acquired resistance determinant. The occurrence of individual species per farm reflected the level of antibiotic use; e.g. novel Taxon 7506 and Taxon 7509 were associated with farms with no antibiotic use.

Conclusions: Our results revealed a high taxonomic diversity of acinetobacters in cattle feces, including an unexpectedly high proportion of potential novel species.

Session 2 - Genetics and Evolution (GE)

O2-1 Genomic diversity of carbapenem-resistant Acinetobacter baumannii reveals distinct virulence evolution

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Carbapenem-resistant A. baumannii (CRAB) ranks among the highest priority pathogens by the US CDC and the WHO Its virulence vary in different geographic locations which is believed to be related to its genetic plasticity. The most common clonal complexe worldwide is ST2. A recent wide-scale analysis of a large collection of 246 A. baumannii serious infections causing isolates from three Mediterranean countries (Israel, Greece and Italy) found both ST2 and ST3 were common. Here, we assessed the diversity of virulence factors of these isolates. Whole genome sequencing revealed many differences in the genomic content of ST2 and ST3, specifically, in antibiotic resistance and capsular genes. We found that ST3 is largely homogenous. In contrast ST2 strains differ greatly in antibiotic resistance genes, KL and OCL types, virulence factors and plasmid content. We found that this diversity is explained by distant evolutionary events; ST2 consists of 8 evolutionary clades (2A - 2H) that greatly differ from each other, however are within clade homogenous. We found high diversity in bap gene (the main gene encoding the biofilmassociated protein BAP) among ST2 isolates. This gene was missing among ST3 isolates, which produced less biofilm than ST2. We found that ST3 isolates harbor a unique Ig-like protein, with an N terminus highly similar to bap. Our results indicate that variability in virulence and resistance mechanisms of CRAB isolates, is explained by distant divergence of ST and clades rather than by more recent lateral gene transfer. Introduction and spread of a new clade into a hospital or even geographical area may result in change in epidemiology and clinical manifestations.

O2-2 Structure and regulation of the hemO gene cluster for heme uptake in Acinetobacter baumannii

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Background: The scarcity of available iron in human fluids encountered by pathogens during infection is part of a non-specific defense referred to as nutritional immunity. Most of the iron in the human body is stored in the heme-prosthetic group, primarily in hemoglobin. Acinetobacter baumannii possess two heme acquisition systems, namely HemT and HemO. The hemO cluster is composed of two operons and two monocistronic genes. One operon consists of two genes encoding an extracytoplasmic function (ECF) sigma factor and an anti-sigma factor, while the second one is composed of four genes coding for the hemophilin secretion modulator (hsmA), a TonB-related protein, a heme oxygenase, and a hypothetical protein. The monocistronic genes are the TonB-dependent receptor, hphR, and the hemophilin, hphA (Bateman et al., 2021. doi: 10.1038/s41467-021-26545-9).

Methods: Functional and gene expression analyses were carried out to characterize the structure and regulation of the hemO gene cluster. Bidirectional deletion analysis of the hemO cluster was performed to identify the minimal region essential for heme uptake. The hemO gene expression was analyzed in several laboratory media and biological fluids. Finally, the contribution of the hemO cluster in the A. baumannii virulence was assessed in Galleria mellonella.

Results: The deletion analysis of the hemO cluster allowed us to define that the TonB-related protein, the heme oxygenase, and the hypothetical protein are not essential for the transport of heme-mimetic GaPPIX. We demonstrated that the expression of the sigma factor, the hemophilin receptor (hphR), and hemophilin (hphA) genes is iron-regulated, and that all these genes are expressed when A. baumannii grows in biological fluids such as human serum, saliva, and urine. We also demonstrate that ECF sigma factor is required for the expression of hphR, hphA, and hsmA. Finally, we observed that the hemO cluster significantly contributes to A. baumannii lethality in the G. mellonella larvae infection model. Conclusions: Our results demonstrated that iron scarcity represents a cue that triggers the expression of ECF sigma factor, which directs the expression of the hemO gene cluster. We also highlight the crucial role of the hemO cluster in the A. baumannii virulence.

O2-3 Genetic manipulation of A. baumannii-infecting bacteriophages to tackle a larger range of capsular types

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Acinetobacter baumannii is the major cause of multidrug-resistant infections. Phage therapy is considered a promise alternative to replace or complement antibiotic usage. A. baumannii displays a high variety of capsular types (K-types) for evading host defenses and protecting themselves from predators. Therefore, A. baumannii-infecting bacterio(phages) evolved to recognize specific Ktypes. This specificity is instigated by capsular depolymerases (DPO), enzymes that are present in 62% of all A. baumannii genomes and that specifically recognize and degrade bacterial capsules allowing these phages to overcome this barrier and proceed with the infection. Our goal is to generate engineered phages against A. baumannii by adjusting their specificity through changing their DPO.

We isolated and cloned novel DPOs from Acinetobacter phages, ending with an in-house collection of enzymes targeting 10 K-types (KL1, KL2, KL9, KL19, KL30, KL32, KL38, KL44, KL45, KL67). DPOs were later expressed and tested for polysaccharide degrading activity. These allows us to genetically engineer phages with altered host specificities. For instance, the native DPO of K38 phage (a Friunavirus that specifically infects K38), was replaced by homologous recombination to harbor K67 DPO. The DPO swapping was confirmed by WGS and the lytic spectra of the engineered phage was characterized against a panel of different K-type strains.

Overall, the collection of available DPOs was expanded to 17 K-specific DPO. Moreover, we demonstrated the implementation of genetic manipulation tools to tune the host range of A. baumannii. The engineered phage equipped with K67 DPO was able to infect strains belonging to this K-type and lost its infectivity against the original host (K38).

In sum, we have a library of DPO available to switch in the well-characterized K38 phage and we were able to prove the benefits of engineering native phages to tackle different K-types. This is highly important to advance the prospect of the application of these phages to manage infections caused by this pathogen, and to generate a platform with exchangeable DPO to control a larger range of A. baumannii strains.

O2-4 Induction and evolutionary conservation of natural transformation in Acinetobacter baumannii

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Background
Acinetobacter baumannii is now well established as a naturally transformable species. Genomic analyses shows high rates of recombination and support a major role of natural transformation in acquisition of antibiotic resistance genes. Interestingly, several studies have reported large variations in the capacity of A. baumannii strains to undergo natural transformation. This could be to strain-specific properties and/or suboptimal experimental conditions to trigger natural transformation.

Methods
In order to facilitate the study of natural transformation, we designed a new method to experimentally quantify transformation and which alleviates the limitations of the selection-based conventional assay. The new method proved accurate and more reproducible than conventional selection-based plating assays. It allows for the first time to quantify the trait of natural transformability in a throughput format with unprecedented accuracy.

Results
We first used the method to identify the inducing signal present in previously reported transformation conditions. Indeed, for many years, agarose-solidified media have been used to induce natural transformation, with the assumption that the solified surface acts as a trigger. Invalidating this hypothesis, and through testing hundreds of transformation conditions, we found that the induction is caused by a soluble compound. This helped us refined the transformation conditions, which can be harnessed for genetic engineering. The refined transformation conditions, and the new transformation assay, allowed us to robustly quantify natural transformation in a diverse population of several hundred isolates of A. baumannii. Results show that the majority of isolates are capable of natural transformation. Yet, extended variations in natural transformation over short phylogenetic distance are observed.
Conclusions
Technical developments allowed quantification of natural transformation in a large collection of isolates, indicating that this is a fast-evolving trait. The evolutionary cause will be discussed.

Session 3 - Bacterial Infections (Inf)

O3-1 Dietary zinc deficiency compromises immunity to Acinetobacter baumannii pneumonia

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Zinc deficiency affects approximately one third of the global population and is estimated to contribute to 1 in 6 cases of pneumonia. In the United States, intensive care unit patients are at increased risk for both zinc deficiency and infection by Acinetobacter baumannii. A. baumannii is a leading cause of ventilator associated pneumonia and a critical public health threat due to increasing rates of multi-drug resistance. Determining the role of host and A. baumannii metabolism during infection has the potential to identify new therapeutic targets to support host immunity and inhibit essential bacterial processes. To test whether dietary zinc deficiency contributes to A. baumannii pathogenesis, we established a murine model of dietary zinc deficiency and A. baumannii pneumonia. In this model, zinc deficient mice have increased A. baumannii burdens and suffer significantly higher mortality. During infection, zinc deficient mice produce more proinflammatory cytokines, including the type 2 cytokine IL-13 that is typically associated with asthma and parasite infection. Antibody-mediated IL-13 neutralization helped protect from mortality and administration of recombinant IL-13 was sufficient to promote dissemination, suggesting that IL-13 is critical for increased susceptibility to A. baumannii pneumonia in zinc deficient mice. To understand the bacterial response to host zinc restriction, A. baumannii genes important for survival in zinc deficient mice were identified using transposon sequencing (Tn-seq). This analysis identified key A. baumannii metabolic pathways as important during infection of the zinc deficient host, including purine biosynthesis. Together, these results indicate that dietary zinc deficiency leads to host immune dysregulation and failure to control bacterial replication, which demand additional metabolic requirements of A. baumannii.

O3-2 Elucidating the role of Acinetobacter calcoaceticus in promoting intestinal inflammation

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Background: Inflammatory bowel disease (IBD), which encompasses the subsets Crohn's Disease and Ulcerative Colitis, is a life-long condition characterized by chronic inflammation of the gastrointestinal tract. Analysis of 3,853 publicly available houses RNA-Seq datasets from 26 independent studies revealed that Acinetobacter calcoaceticus was one of the top 10 highest elevated bacteria in Crohn's Disease patients. Acinetobacter are well-characterized by their antibiotic resistance, but little is known about its relationship to the intestine. We hypothesize that A. calcoaceticus colonizes the gut and exacerbates intestinal inflammation. Materials & Results: To examine the ability of A. calcoaceticus to grow in the gut, we grew commercially available and clinical isolates of A. calcoaceticus in vitro in minimal media with stressors found in the gut. All strains grew in a range of pHs (4-7), osmolarity (0.1-1 M NaCl), ethanol (1- 5%) and hydrogen peroxide (0.05-0.1%); indicating that A. calcoaceticus is well-adapted to withstand the harsher conditions of the gastrointestinal tract. To confirm our in vitro findings, we oral gavaged mice with A. calcoaceticus and examined fecal levels using selective agar. and qPCR 7 days after gavage.

Acinetobacter was observed in low levels in the vehicle control mice and high levels of Acinetobacter in our mice gavaged with A. calcoaceticus (average 9.9x 107 CFU); suggesting that A. calcoaceticus readily colonizes the gut. To examine the ability of A. calcoaceticus to enhance inflammation, we oral gavaged mice with either a vehicle control (PBS) or A. calcoaceticus and the following day we treated mice with 2,4,6-trinitrobenzene sulfonic acid (TNBS) on day 5 to induce colitis. Mice treated with A. calcoaceticus and TNBS lost more weight and had worse histological scores than mice treated with vehicle control and TNBS; indicating A. calcoaceticus worsens intestinal inflammation. Consistent with these findings, addition of live A. calcoaceticus to insideout-intestinal organoids stimulated the production of pro-inflammatory cytokines Tnf, IL-8, IL-1α and Mcp-1; indicating that A. calcoaceticus can drive epithelial inflammation. Conclusions: These data indicate that A. calcoaceticus can promote intestinal inflammation and maybe a contributing factor to the inflammation experienced by IBD patients. We speculate that traditional IBD treatments, such as anti-TNF therapy.

O3-3 Epithelial-macrophage communication promotes clearance of Acinetobacter baumannii from the airway

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Acinetobacter baumannii is an extremely versatile, multidrug resistant pathogen. A prominent nosocomial as well as community-acquired pathogen, it has a very high mortality rate of up to 35% in pneumonia cases. It has become crucial to better understand how the host senses and responds to A. baumannii infection, and while some innate molecules have been demonstrated to be important in the airway, the specific contribution of the airway epithelium in vivo is currently unknown. A major innate immune transcription factor is NF-κB. We initially observed that its systemic inhibition greatly increased bacterial numbers in the airway and systemically. To determine the role of NF-κB in the airway, we utilized an airway epithelial cell (AEC) specific Rela (p65) knockout mouse (AEC-RelA). At 24 h after infection the AEC-RelA mouse had >300-fold more (p40-fold more ($p<0.001$) in homogenated lung. Both genotypes had marked consolidation of the airways. Early after infection at 4 h there was no difference in bacterial burden but several inflammatory cytokines were decreased, including neutrophil chemokines that were confirmed by qRT-PCR and reduced cell numbers by flow cytometry. However, complementation with recombinant chemokines did not restore bacterial clearance. We further investigated the AEC differences by RNA-seq and identified several pathways including: reactive oxygen species (ROS) production in macrophages, iNOS and pathogen-induced cytokine signaling that were reduced in the AEC-RelA mouse. Based on the RNA-seq data, we quantified ROS production in the cells found in the airway by flow cytometry. ROS production in alveolar macrophages was found to be reduced by 35% ($p<0.05$) in the AEC-RelA mouse. These data suggest that production of NF-κB in AEC is important for controlling A. baumannii in the airway, potentially through coordination of killing activity in alveolar macrophages.

O3-4 Mechanistic insights into the phagocytosis resistance of the WHO priority pathogen Acinetobacter baumannii

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Despite the impressive genetic and phenotypic heterogeneity among A. baumannii isolates, the presence of a key factor, at the crossroad between the resistance and virulence arsenals, is conserved: the polysaccharidic capsule. The capsule is known to be involved in resistance to stresses such as antimicrobials, disinfectants and desiccation, and has also been linked to phagocytosis resistance. The capsule is also a very diverse component of A. baumannii, as demonstrated by the large number of capsule types, encoded by various combinations of genes. The regulation and underlying mechanisms of the capsule production remain to be studied more extensively. Besides the new eukaryotic cellular models to study A. baumannii, such as epithelial cells and macrophages, we propose amoebae as new pratical and environmentally relevant models. Indeed, amoebae as model systems present versatility, robustness and phagocytic capacity under different conditions. Moreover, they are suitable for high-throughput screens and as such will be further investigated in my project.

Hence, the host laboratory has developed and validated the amoeba Acanthamoeba castellanii model and has obtained data on a phagocytosis-induced mucoid phenotype of A. baumannii. This protective mucoidy induction is conserved among 43 relevant clinical isolates and leads to phagocytosis resistance to amoebae, while it is not observed in the grazing-sensitive Escherichia coli. A non-capsulated mutant of A. baumannii, with a deletion of the itrA gene, is sensitive to grazing by A. castellanii. Transmission electron microscopy accompanied by density gradients have confirmed the role of the capsule in this phenotype, showing an increased capsule deposition on phagocytosis-resistant bacteria.
In this project, I will study the mechanisms involved in the induction of this protective phenotype, using the novel cellular model A. castellanii. This model will allow for the high throughput screening of a transposon library, identifying actors involved in the resistance phenotype. Moreover, I will perform a RNA-seq to screen for variations at the transcriptomic level between the bacteria under and without phagocytic pressure. Lastly, biochemical approaches will determine the polysaccharidic composition of the mucoid induced phenotype. This project will yield critical information on the mechanisms involved in capsule production and virulence in human pathogens.

Session 4 - Antimicrobial Resistance (AMR)

O4-1 Phage-mediated transmission of colistin resistance in Acinetobacter baumannii

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Background: The rise of antimicrobial resistance has forced the reintroduction of colistin as a “last-resort” drug to treat Acinetobacter baumannii infections, leading to the emergence of colistinresistant strains. The most frequent mechanism of colistin resistance in A. baumannii clinical isolates relies on modifying the lipid A moiety of LPS by adding the phosphoethanolamine (PetN) group, which prevents colistin binding to the outer membrane [1]. In this study, we investigated the role of the Φ19606 bacteriophage as a vehicle of colistin resistance.

Methods: We collected worldwide A. baumannii whole-genome sequencing data from PubMLST to evaluate the Φ19606 dissemination. Different approaches were employed to evaluate the costs and benefits of Φ19606 presence in terms of fitness, colistin resistance, and expression of the phage gene eptA1 involved in lipid A phosphoethanolamination in a panel of A. baumannii strains. Results: Φ19606 was more frequently detected in A. baumannii strains belonging to sequence type 2, and could horizontally be transferred between A. baumannii strains. Strains harboring Φ19606 showed increased colistin tolerance and higher frequency of spontaneous mutation to colistin resistance than the phage-free parental strains. The colistin-tolerant phenotype was due to the presence of the eptA1 gene in the Φ19606 genome. The eptA1 gene shares 95% identity with the chromosomal pmrC gene of A. baumannii, and both genes encode for lipid A PetN transferase enzymes. Under conditions of controlled gene expression, EptA1 was more efficient than PmrC in lipid A phosphoethanolamination, determining a dramatic increase in colistin resistance concomitant with loss of A. baumannii fitness in the Galleria mellonella larvae model of infection. Figure 1 summarizes the obtained results.

Conclusions: A novel model of phage-mediated dissemination of colistin resistance is demonstrated. By directing the horizontal transfer of the eptA1 gene, Φ19606 represents the first example of a self-transmissible phage driving colistin resistance. Our findings emphasize the need to monitor bacteriophages as possible spreading vectors of polymyxin resistance genes.

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O4-2 Resistome and Virulome of cefiderocol resistant Acinetobacter baumannii clinical isolates from Alexandria, Egypt

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Background

Carbapenem resistant A. baumannii is of high concern in clinical settings due to the few therapeutic options available, causing a high rate of fatal infections worldwide. Cefiderocol is one of the last resort antibiotics although resistant isolates are being reported. A combination of mechanisms appears to be responsible for resistance to cefiderocol. This study aimed to analyse the genetic features of eight cefiderocol resistant A. baumannii clinical isolates from Alexandria, Egypt.

Methods

Eight cefiderocol resistant isolates obtained from hospitals from Alexandria were studied. We performed the following techniques: 1) Determination of Minimum Inhibitory Concentrations by VITEK2® and cefiderocol susceptibility testing by disk diffusion method; 2) Whole Genome Sequencing by Illumina Miseq technology; 3) Multi Locus Sequence Typing Pasteur Scheme; 4) Resistome analysis by TORMES pipeline against Resfinder, CARD and ARG-ANNOT databases; 5) Virulome analysis by Virulence Factors Database; 6) Sequence alignments using MEGA11; 5) Plasmid analysis by commercial kit and Pulsed Field Gel Electrophoresis with S1 endonuclease.

Results

Resistance profile of the isolates is shown in Table 1. Five isolates belonged to International Clone (IC) 2 [ST2, ST570, ST600] and three isolates belonged to IC7 (ST113), IC8 (ST613) and IC9 (ST85), respectively. Co-existence of several blaOXA-type (blaOXA-66, blaOXA-64, blaOXA-68, blaOXA-94 and blaOXA-23), blaADC-like, blaTEM-1, blaGES-11, blaNDM-1 and blaPER-7 genes was detected. Genes conferring resistance to aminoglycosides, macrolides, tetracyclines, chloramphenicol, sulfonamides, rifamycin and trimethoprim were encountered. Virulence factors responsible for adherence, biofilm production, Type II and VI secretion systems, exotoxins, exoenzymes, immune modulation and iron uptake were present. Mutations in ftsI gene (PBP3) were found in five isolates. Plasmids ranging in size from 1.7 to 44.1 Kb were also detected.

Conclusions

High antimicrobial resistance ratios were found. A high clonal diversity was encountered among cefiderocol resistant isolates. The isolates harboured a wide variety of genes mediating resistance to most classes of antibiotics and multiple virulence factors. Mechanisms previously described to be responsible for cefiderocol resistance were found, such as the presence of blaNDM-1 and blaPER, or mutations in the ftsI gene.

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O4-3 Transfer of the chromosomally encoded OXA-23 carbapenemase by recombination in Acinetobacter baumannii

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Background: OXA-23 is the most common acquired class-D carbapenemase in Acinetobacter baumannii. The spread of OXA-23 has been linked to diverse transposons, e.g., Tn2006 or Tn2008. We aimed to investigate the transfer of a chromosomally encoded blaOXA-23 in a clinical A. baumannii isolate. Materials/methods: Broth mate experiments were performed using the MC75 carbapenem-resistant isolate (blaOXA-23-positive) as donor and the rifampicin-resistant BM4547 as recipient. Selection was performed using rifampicin (100 mg/L) and ticarcillin (150 mg/L). Transconjugants were screened by PCR for the presence of blaOXA-23. Whole genome sequencing was performed (MiSeq and MinION platforms) followed by cgMLST/accessory typing (Ridom) and MLST (Pasteur) analysis. MICs were determined using the agar dilution method and interpreted using the EUCAST breakpoints (v 13.0). Genomes were aligned using progressive Mauve and confirmation of recombination spots was performed using k-mers analysis. Results: The donor MC75 (ST15) encoded the intrinsic blaOXA-51 variant and the blaOXA-23 in Tn2008 embedded in the chromosome. The recipient strain BM4547 was ST126 and harboured the OXA-51 variant blaOXA-64 and had no acquired carbapenemase. Mating out assays resulted in BM4547::blaOXA-23, which had the same ST and intrinsic blaOXA as the recipient and had acquired the blaOXA-23 in the chromosome. By antimicrobial susceptibility testing the BM4547:: blaOXA-23 had increased MICs only for imipenem and meropenem. Genome analysis revealed that a 17kb region including blaOXA-23 was mobilised by recombination from the donor to the recipient (Figure 1). Furthermore, additional recombination events between the donor and the recipient were identified resulting in 48 alleles difference between BM4547 and BM4547::blaOXA-23. MC75 and BM4547::blaOXA-23 differed in 2484 alleles in cgMLST/accessory analysis.
Conclusions: Our data have not only shown transfer of the chromosomally encoded blaOXA-23 by recombination, but also identified a greater number of recombination events beyond the carbapenemase. These data highlight that recombination events contribute to the spread of antimicrobial resistance, but can also result in high genetic diversity by shuffling the bacterial genome and leading to differences in the genetic makeup of a bacterial isolate. The consequence of recombination events in tracking bacteria during outbreaks needs to be assessed.

O4-4 Increased Intraspecies Transfer of blaCTX-M-1 After Interspecies Acquisition from Salmonella enterica to Acinetobacter baumannii by Natural Transformation

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Background: Dissemination of antimicrobial resistance is facilitated by horizontal gene transfer of resistance determinants between bacterial cells. The emergence and spread of blaCTX-M genes, which confer resistance to the majority of beta-lactam antibiotics, poses a significant threat to public health. These genes are commonly found in clinical Enterobacterales, and acquisition of plasmidcarrying resistance genes by conjugation is well-established while the role of natural transformation in their dissemination is less known. This study aims to evaluate the involvement of natural transformation in blaCTX-M genes dissemination. Methods: Genomic DNA of the foodborne isolate Salmonella enterica serovar Typhimurium Sal25 carrying blaCTX-M-1 was used as donor DNA and A. baumannii A118 as recipient cell in transformation during motility on wet surfaces. A. baumannii ACI transformant, which acquired blaCTX-M-1, was used as donor DNA in further transformation assays using A. baumannii A118, A. baylyi BD413, A. nosocomialis 013 and Acinetobacter sp. 065 as recipient cells. Transformants were screened by selective plating with cefotaxime 30 µg/mL, and antimicrobial susceptibility phenotype and genotype was confirmed by disk diffusion and broth microdilution methods and PCR, respectively. Transformation frequency was determined as the number of transformants per recipient cell. Results: A. baumannii A118 was successfully transformed with Sal25 DNA at a frequency 2.7x10-8; however, acquisition of blaCTX-M-1 was only verified in 1 transformant, named ACI. This gene was successfully transferred to A118 in subsequent transformation events at a frequency of 1,8x10-6, with all tested transformants being positive for the blaCTX-M-1 acquisition. No transformants were obtained using the other species tested as recipient. All the transformants were resistant to cefotaxime, amoxicillin and aztreonam, according to the genotype transferred, with MIC values >512 mg/L for cefotaxime. Conclusions: It is demonstrated that blaCTX-M-1 gene can be horizontally transferred by natural transformation between genetically divergent species. Although the first event occurs at a low transformation frequency, once acquired this gene can easily spread among genetically similar bacteria after homologous recombination. Overall, natural transformation contributes to the spread of clinical important resistance genes in bacteria that might not be of clinical origin, highlighting that this mechanism should be studied from One Health perspective.

Session 5 - Pathogenicity (P)

O5-1 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-usher pathway. To gain insight into this process, we performed structural and functional studies of the Csu system. The Xray structure of the CsuC-CsuE chaperone-subunit pre-assembly complex revealed the basis for bacterial attachment to abiotic surfaces (1). 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. The 3.4 Å resolution cryo-electron microscopy structure of the Csu pilus rod revealed that in contrast to the thick helical tubes of the classical type 1 and P pili, archaic pili assemble into a conceptually novel ultrathin zigzag architecture secured by an elegant clinch mechanism (2). The molecular clinch provides the pilus with high mechanical stability as well as superelasticity, a property observed now for the first time in biomolecules, while enabling a more economical and faster pilus production. Furthermore, we demonstrated that clinch formation at the cell surface drives pilus secretion through the outer membrane. These findings suggest that clinch-formation inhibitors might represent a novel strategy to fight multidrug resistant bacterial infections.

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O5-2 Epinephrine exposure facilities the adaptation of Acinetobacter baumannii to hostile host environments and concomitantly promotes the polymicrobial interactions

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The high environmental adaptation of Acinetobacter baumannii makes it one of the most successful pathogens in the modern healthcare system. Understanding how this pathogen copes with the human host and determining the environmental factors influencing A. baumannii incidence is imperative in developing novel prevention and treatment strategies. In this study, we examined the impact of Epinephrine (Epi), a stress hormone, on the physiological characteristics of A. baumannii. In the presence of Epi from 10 µM, we observed a marked stimulation in the growth and biofilm formation of A. baumannii. Using a proteomic approach and phenotypic validation, we believe that A. baumannii can use Epi as a pseudo-siderophore in the early stages of contamination. In a mixed A. baumannii and S. aureus biofilm model, our findings demonstrated that the Epi exposure caused a shift in the ratios of bacterial species, resulting in a change from a predominance of Gram-positive bacteria to Gram-negative bacteria. In addition, the presence of Epi potentially favored the motility of S. aureus, a virulence factor that is not often developed in this species.

O5-3 Intracellular Acinetobacter baumannii in vivo: potential roles in UTI and respiratory infections

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The antibiotic-resistant bacterium Acinetobacter baumannii is a leading cause of hospitalassociated infections. Despite surveillance and infection control efforts, new A. baumannii strains are regularly isolated from health care facilities worldwide. We and others have recently shown that A. baumannii new clinical isolates can persist and replicate in macrophages and epithelial cells. However, the relevance of an intracellular lifestyle during infection is still unknown. In a mouse model of urinary tract infection, up to two months after the resolution of A. baumannii infection, inserting a catheter into the bladder of mice with resolved infection led to the resurgence of a samestrain urinary tract infection in ~53% of the mice within 24 hours. We identified intracellular A. baumannii in the bladder epithelial cells of mice with resolved infection, which we propose could act as a host reservoir that was activated upon insertion of a catheter, leading to a resurgent secondary infection. Moreover, in a murine acute respiratory infection model, modern A. baumannii clinical isolate 398, but not the common lab strain 19606, can infect alveolar macrophages. The in vitro characterization of the Acinetobacter containing vacuole (ACV), showed that both strains interacts with the endocytic pathway, as indicated by EEA1 and LAMP1 markers; however, the fate of these strains diverges at a later stage. While 19606 interacts with the autophagic pathway and is then eliminated by the macrophage, 398 replicates in ACVs and are not degraded. We show that 398 reverts the natural acidification of the phagosome by secreting large amounts of ammonia, a byproduct of amino acid catabolism. We propose that this ability to survive within macrophages may be critical for the persistence of clinical A. baumannii isolates in host cells during infection.

O5-4 Desiccation induces apparent death in the pathogenic bacterium Acinetobacter baumannii

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Background: Acinetobacter baumannii is endowed with an extraordinary ability to withstand desiccation. This trait, combined with the ability to survive in the absence of nutrients, is supposed to facilitate the persistence and dissemination of A. baumannii in healthcare settings (Zeidler and Mueller, 2019. Doi:10.1111/1462-2920.14565). While the long-term survival of A. baumannii on dry surfaces has a negative impact on infection control measures, the mechanisms at the basis of its resistance to desiccation are still elusive.

Methods: The adaptive response to long-term desiccation under conditions resembling the hospital environment (20.88 ± 0.59 °C, 13.00 ± 5.58% relative humidity) was investigated in two A. baumannii reference strains, namely the type strain ATCC 19606T and the prototypic epidemic ST2 strain ACICU. Culturability, morphological changes, metabolic adaptation, and virulence potential of A. baumannii cells before and after desiccation stress were evaluated. Transcriptomic analysis was also performed to unveil the molecular mechanisms involved in the response to desiccation stress. Results: During desiccation, both strains endure dramatic structural and functional impairments consisting of a progressive decrease in cellular volume, loss of membrane integrity, and reduction in metabolic activity. This reflects in a dramatic decrease in culturability and virulence in the Galleria mellonella larvae model of infection. Notably, single-cell analyses revealed the existence of bacterial sub-populations capable of switching to a viable but nonculturable (VBNC) state after desiccation. Transcriptomic analyses substantiated the entrance in a dormancy state by showing an enhancement of metabolic flux to the glyoxylate shunt pathway, responsible for bacterial quiescence (Rittershaus et al., 2013. Doi: 10.1016/j.chom.2013.05.012). Resuscitation experiments revealed that VBNC cells can repair membrane damage, restore their cellular volume, and completely recover the culturable state and virulence after rehydration in isotonic buffers or in biological fluids.

Conclusions: Desiccation-induced VBNC bacteria may constitute a reservoir of apparently dead but potentially clonogenic and virulent cells, which impose a paradigm shift for evaluating bacterial desiccation resistance. Our findings suggest that culture-based assessment of A. baumannii viability may fail to detect VBNC sub-populations induced by dehydration and consequently underestimates the number of cells able to generate a progeny on conventional culture media.

Session 6 - Prevention and Treatment (PT)

O6-1 Targeting multidrug-resistant Acinetobacter baumannii to develop nextgeneration antibiotics

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Global health crises caused by microorganisms have changed life as we know it. The rise of antibiotic-resistant bacteria pose another imminent threat by making diseases that were once treatable, lethal again. At the top of the critical pathogens list, as denoted by both the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC), is carbapenemresistant Acinetobacter baumannii. The human pathogen earned this place due to a dangerous combination of characteristics: (i) facile acquisition of drug-resistance genes resulting in multidrug (MDR)- to extensively and even pandrug-resistant strains reported worldwide, and (ii) the ability to resist disinfectants and prolonged periods of desiccation. This has enabled A. baumannii to thrive in clinical settings while existing treatments become inadequate and few new drugs are in the pipeline. In this project, we developed a method to specifically target a variety of A. baumannii strains. In this method, we generated an unbiased library of camelid-derived, variable antibody domains (nanobodies/VHH) targeting the entire cell surface of A. baumannii. From this library, we could select nanobodies capable of binding a variety of living A. baumannii strains. We are using these binders to (i) obtain new insights in surface-exposed elements of this infamous bacterium, and (ii) fuse them to several ‘effector’ proteins and chemical compounds to investigate their potential diagnostic as well as therapeutic antimicrobial, anti-virulence and/or anti-resistance use. By targeting this top priority, MDR bacterium in a highly specific manner, this project provides a proof-of-concept for targeting human pathogens. This will ultimately aid in the development of new, next-generation antibiotics.

O6-2 OXA-23 β-lactamase Overexpression in Acinetobacter baumannii results in cellular vulnerabilities that can be targeted for new drug development

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Multidrug resistant (MDR) Acinetobacter baumannii is a global health menace responsible for approximately 2 million infections and 450,000 deaths annually worldwide. In particular, A. baumannii has become increasingly difficult to treat due to antibiotic resistance mechanisms such as β-lactamase expression, which is a major mechanism responsible for resistance to penicillins, cephalosporins, and carbapenems in MDR A. baumannii. In fact, stable high-level expression of at least one β-lactamase has been rapidly increasing and reported to occur in up to 98.5% of modern clinical A. baumannii isolates. Moreover, the OXA β-lactamase is universally present in the A. baumannii chromosome, suggesting it may have a yet-to-be-identified cellular function beyond antibiotic resistance. We have previously shown that OXA-23 β-lactamase overexpression in A. baumannii drives peptidoglycan composition alterations, reveals conditionally essential vulnerabilities, and results in antimicrobial susceptibility changes. Consequently, we predicted that these collateral changes to A. baumannii physiology due to OXA-23 overexpression would result in new antimicrobial targets. Using a Selleck FDA-approved library as proof of principle, we identified Fendiline, a calcium channel blocker, had significantly more activity against OXA-23 overexpressing cells compared to uninduced cells. Follow-up studies revealed that Fendiline is bactericidal and has potential as a broad-spectrum gram-negative antimicrobial. Our results highlight that b-lactamase overexpression in A. baumannii creates new genetic vulnerabilities, which may represent novel targets for antimicrobial agents specific to MDR A. baumannii and other β-lactamase overexpressing Enterobactericeae, while having no impact on the normal flora.

O6-3 Bacteriophage susceptibility in Acinetobacter baumannii – Capsule type as prerequisite to predict phage lysis?

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Background: Therapeutic use of bacteriophages is a promising approach in the fight against carbapenem-resistant Acinetobacter baumannii (CRAB). Previous studies consider the capsule type (CT) an important factor for phage susceptibility.

Methods: Out of 317 whole-genome sequenced clinical CRAB obtained from different countries between 2012 and 2016, 26 were chosen based on perfect or very high CT match confidence (100% vs. ≥99% coverage, 100% vs. ≥95% nucleotide identity) according to Kaptive database (DOI: https://doi.org/10.1128/JCM.00197). The host range of 17 A. baumannii phages was determined for these isolates using the direct spot testing method and experiments performed in triplicate. Five additional CRAB isolates identified by cgMLST similarity search (<20 alleles difference considered same cgMLST cluster) based on the broadly phage susceptible isolate ABC033, had their phage host range analyzed as described above. Here, the CT match confidence was lower (87%-93.5% coverage, 93.3-99.96% nucleotide identity).

Results: In total 31 CRAB with 12 different CTs were analyzed, with K6 (n=5), K12 (n=4) and K23 (n=3) as most frequent. Four capsule loci (KL) couldn't be associated to a known CT. Five of the CTs were found in ≥2 CRAB isolates. 20/31 isolates were sequence type (ST)2 according to Pasteur scheme. Nine isolates did not show susceptibility to any tested phage. Some CTs were associated with broad phage susceptibility, while isolates with the same CT also belonged to the non-susceptible group (e.g., ABC297, ABC217). Isolates identified using similarity search were associated with K2 (n=3) and KL213, unknown CT (n=2) and showed comparable susceptibility patterns. Six phages, all myoviruses, lysed ≥ 7 CRAB isolates and presented comparable host range with preference towards ST-2 while not being restricted to a single CT. In general, myoviruses showed a broader host range compared to sipho- and podoviruses, which were very specific for certain isolates. Results are summarized in Figure 1. Conclusion: Our data confirm that the A. baumannii CT has an impact with regard to phage susceptibility, however the CT is not the only prerequisite to predict phage susceptibility. Also, our results highlight that bacteria within the same cgMLST cluster are likely to be lysed by the same phage(s).

O6-4 To Combat Drug-resistant Bacteria from a Chemist's Point of View

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The problem of bacterial resistance is becoming more and more serious around the world. The development of new antibiotics is not as soon as the mutation of bacteria. The overuse and misuse of antibiotics are major contributing factors to the resource of antibiotic-resistant bacteria; the abuse of antibiotics has led to the emergence of superbugs. We first used mass spectrometry to identify the pathogen, and next used mass spectrometry to identify post-translational modifications of the proteome to identify drug-resistant bacteria. However, knowing the species of bacteria is not the most serious problem in medicine. The most pressing work is prevention, disinfection, and treatment. We used visible blue light and natural photosensitizer (curcumin) for photodynamic antibacterial and cooperated with organic chemists to synthesize a series of curcumin analogs with blue light photosensitive function. This structural modification increased its bactericidal efficacy and achieved good results. Finally, the bioinformatics proteome analysis was to be used to understand the mechanism of photodynamic antibacterial. Overall, our approach to combating drug-resistant bacteria is should be promising, and further research and clinical trials are necessary to evaluate the safety and efficacy of this research.

Session 7 - Physiology and metabolism (FisMet)

O7-1 Light Signal Transduction and Chronobiology in the Human Pathogen Acinetobacter baumannii: Role of the BfmRS two component system

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Acinetobacter baumannii belongs to the ESKAPEE group due to its ability to "escape" antibiotic treatment and this pathogen is associated with high morbi-mortality infections [1]. In this context, the World Health Organization included A. baumannii within the critical group of bacteria that constitute serious threats to human health [2].

We have extensively shown that A. baumannii perceives and responds to light, modulating global aspects of its physiology. Most interestingly, light modulates its persistence in the environment, antibiotic susceptibility and virulence [3]. We postulate that these bacteria could respond to light to synchronize their physiology with the host, to optimize infection outcome.

In this work, we demonstrate that the expression of the blsA gene presents oscillations along the day, when cells are entrained in light-dark cycles of 12 h each (12L: 12D) at 23°C. These oscillations persisted when the cultures were further incubated under constant darkness. These observations are compatible with two criteria that define a circadian rhythm: free-running and entrainment. On the other hand, we have recently revealed that the BfmRS two-component system is directly involved in light signal transduction at 23 and 37°C. In particular, we observed that light regulates motility in A. baumannii V15 strain at 37°C. This light regulation is completely lost in the ΔbfmRS mutant, a phenotype that depends on both BfmR and BfmS, which present antagonistic behavior [4]. The BfmR phosphrylatable form (BfmR~P) is the one involved in motility inhibition, and BfmS is a phosphatase for BfmR~P. The working model postulates that BfmR could be a motility repressor in its light-phosphorylated state, while BfmS would mediate its dephosphorylation in dark. Sequence comparisons revealed that BfmR is 42.93% identical to RpaA, a response regulator involved in the circadian clock in Cyanobacteria [5]. We hypothesize that BfmRS could be a component of a circadian clock that integrates environmental signals, including light. BfmS could antagonize BfmR function in light or dark, and let it function in the other condition, as occurs in the cyanobacterial KaiABC system.

These results could contribute to establish a new paradigm, with potential impact on biomedicine, since the current evidence regarding the circadian rhythms in nonphotosynthetic prokaryotes is scarce.

O7-2 Molecular interactions between peptidoglycan integrity maintenance and outer membrane lipid asymmetry in Acinetobacter baumannii

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The Gram-negative cell envelope is essential because it provides mechanical strength to counter the turgor and acts as a barrier to restrict the entry of toxins and antibiotics. The outer membrane (OM) and peptidoglycan are tightly linked during growth, but the molecular factors and pathways that coordinate assembly are poorly understood. Identifying cooperative factors that promote assembly between cell envelope layers may provide insights into how we can effectively target antimicrobials against Gram-negative pathogens. Acinetobacter baumannii is a nosocomial pathogen that has a high propensity to overcome antimicrobial treatment. LdtJ is a periplasmic LDtranspeptidase that promotes fitness during growth in A. baumannii. However, it is unknown how LdtJ enzymatic activity contributes to growth. Previous work in Escherichia coli showed that periplasmic tetrapeptide accumulation is toxic. Muropeptide analysis of ΔldtJ showed increased tetrapeptide pools, which are LdtJ substrates, and this accumulation may also be toxic in A. baumannii. Transposon sequencing analysis suggested that deletion of mla genes in ΔldtJ background restored the growth defect. Maintenance of lipid asymmetry (Mla) retrograde phospholipid transport system removes mis localized surface-exposed phospholipids to maintain OM asymmetry. When Mla is disrupted, accumulation of surface-exposed phospholipids induces OM vesicle formation. Here, we tested the hypothesis that periplasmic tetrapeptide toxicity can be relieved by mla disruption, where increased OM vesicle formation may release tetrapeptides into the environment to reduce cellular toxicity. Thus, mla serves as a compensatory mutation to restore ΔldtJ fitness defect in growth. This study will provide insights into how OM asymmetry and peptidoglycan integrity maintenance pathways are coordinated to maintain cell envelope homeostasis.

O7-3 Systematic Dissection of Genetic Vulnerabilities in Acinetobacter baumannii

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Addressing antibiotic resistance in A. baumannii requires a comprehensive understanding of its vulnerabilities, particularly in essential genes. These genes are the current and likely future targets of antibiotics, but they remain understudied. In this work, we employ CRISPR interference (CRISPRi) technology and machine learning to systematically phenotype essential genes, identifying genetic vulnerabilities that affect growth and antibiotic susceptibility for potential exploitation in future mono- or combination therapies.

To gauge the importance of essential genes and pathways in A. baumannii physiology, we measured the loss of fitness following gene knockdown across a broad range of reduced expression levels. By modeling the relationships between knockdown efficiency and phenotypic consequences, we identified key vulnerabilities. Among the most vulnerable genes, we found established antibiotic targets (cell wall) and less explored potential targets. Notably, genes involved in oxidative phosphorylation, especially those encoding the NADH dehydrogenase I complex (NDHI or nuo), emerged as unique genetic vulnerabilities. As a resource, we provided a ranked set of genes and pathways vulnerable to knockdown for improved target prioritization in combination with biochemical and structural data.

To identify essential genes that modulate the efficacy of existing drugs, we screened our CRISPRi library against last-resort antibiotics. In addition to synergies between antibiotics and knockdowns of their known targets, we found a plethora of unexpected gene-antibiotic interactions that impact antibiotic function. For example, perturbation of tRNA charging pathways increased resistance to carbapenem antibiotics, and knockdown of genes encoding NDH-I increased colistin sensitivity. We also observed an anticorrelated pattern of antibiotic-gene interactions in colistin and rifampicin that may explain the known synergy between the two drugs. In general, we suggest that drug-gene synergies and antagonisms found in our screens can inform future combination therapies.

Our integration of CRISPRi technology with systems biology demonstrates the power of unbiased approaches to uncover essential gene and antibiotic functions in A. baumannii. By identifying genetic vulnerabilities and enhancing our understanding of antibiotic function, we aim to contribute to the development of more effective therapies.

O7-4 Identification and characterization of a novel pathway for aldopentose degradation in Acinetobacter baumannii ATCC 19606

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Background: Acinetobacter baumannii is an emerging opportunistic pathogen that is a significant cause of nosocomial infections. The emerging antibiotic resistances of the pathogen but also its high desiccation resistance have contributed to its success. Another factor contributing to the adaptation to the human host is its broad metabolic versatility, such as using different amino acids, aromatic compounds, fatty acids, alicyclic compounds, alcohols, and sugars as carbon and energy source. Information concerning sugar metabolism and its link to pathogenicity is very limited.

Methods: Transcriptomics, mutant studies, growth studies, enzyme activities.

Results: Transcriptome analyses performed with A. baumannii ATCC 19606 grown on L-arabinose led to the identification of two significantly upregulated gene clusters. Cluster I contains the genes araC1, araD1, and araE1, a potential regulator and importer gene, and cluster II the isogenes araC2, araD2, and araE2, a potential regulator, importer, and two genes of unknown function. araCE encode proteins with high similarities to enzymes that catalyze the oxidation of a pentonate to αketoglutarate which is then funneled into the Krebs cycle. Genes encoding a potential dehydrogenase and lactonase mediating the oxidation of the pentoses to the pentonates could not be identified by transcriptome analyses. However, pentose dehydrogenase activity was observed in cell-free extracts. Deletion of araC1-E1 led to a loss of growth on L-arabinose, whereas deletion of the isogenes abolished growth on D-xylose and D-ribose. The two genes of unknown function in cluster II are essential for growth on D-ribose, but not D-xylose. The regulation of the two branches differs: regulator I acts as a repressor and regulator II as an activator. Mutational analyses confirmed the role of the putative importers in L-arabinose (cluster I) or D-xylose and D-ribose (cluster II) transport. Furthermore, we could demonstrate that utilization of L-arabinose supports longterm survival and desiccation resistance.

Conclusions: A. baumannii oxidizes aldopentoses via pentonate to α-ketoglutarate by a novel branched pathway. L-arabonate is degraded to α-ketoglutarate by AraC1, AraD1, and AraE1, and D-xylonate and D-ribonate by AraC2 – AraE2.

Posters

Topics 1 – 4

PO1 | Characterization of 85 Acinetobacter baumannii strains by sequence typing, antibiotic resistance, CRISPR-Cas systems, and phage sensitivity

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Acinetobacter baumannii is an opportunistic pathogen known to cause fatal nosocomial infections worldwide. It has been ranked as of critical priority by the WHO due to its ability to rapidly acquire resistance to clinically approved antibiotics, reason why treatment options have become very limited in some cases. Bacterio(phages) are viruses that infect bacteria. Their specificity toward targeted bacterial pathogens, high safety, and replication at infection sites make them a promising alternative or complement for therapy. However, this phage-based approach still presents some challenges such as a narrow host range, possible emergence of phage resistance with or without cross resistance to other antimicrobials, and the host immune response to phages. It is hypothesized that some of these limitations could be lifted by having a better understanding of phage-host interactions.

Here, we assembled a collection of 85 A. baumannii strains and 15 virulent phages. A. baumannii strains were first confirmed by sequencing of the 16S rRNA gene and by amplification of the blaOXA-51-like gene, which was followed by their characterization through i) sequence typing (ST, Pasteur scheme), ii) susceptibility to 17 commercially available antibiotics, iii) presence of type I-F1 and I-F2 CRISPR-Cas systems, and iv) sensitivity to 15 phages. Among these phages, 8 were myoviruses, 4 siphoviruses, and 3 podoviruses.

We found 28 STs among the 85 A. baumannii strains, with ST1 (14.1%) and ST2 (14.1%) being the most prevalent. Four new sequencing types appeared to have also been identified. Antibiotic susceptibility assays revealed that 74% (63/85) of the strains were multidrug resistant, with one strain being resistant to all antibiotics. On average, a given strain was resistance to 7 antibiotics. Of note, 14 strains were sensitive to all antibiotics. CRISPR-Cas analyses showed that 41% (31/85) of the strains harbored one system. Apparent correlations were noticed between ST1/ST81 and the type I-F1 system as well as the type I-F2 and ST52/ST79. Interestingly, 93% (79/85) of our A. baumannii strains were sensitive to at least one phage. No association was found between ST, antibiotic resistance, CRISPR, and phage sensitivity. In the forthcoming months, we will further investigate the interactions between A. baumannii-phage-antibiotics.

P02 | Epidemiology and heterogeneity of genotypes and phenotypes of the A. baumannii strains

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Background: Acinetobacter baumannii is a Gram-negative opportunistic pathogen, displaying resistance to antibiotics, desiccation, and disinfectants. We examined the limitations of the plasticity and heterogeneity of A. baumannii strains along the local and global epidemiological situation.
Methods: We assembled the complete genomes of modern clinical isolates from Belgian hospitals and broadly used strains of A. baumannii. Subsequently, we evaluated genotypes (sequence types [ST], capsule locus [KL] types, outer core lipooligosaccharide locus [OCL] types, antimicrobial resistance genes and virulence genes) and the phenotypes (hemolytic and protease activity, capsule production, capsule thickness, macrocolony morphology, natural competence, and in vivo virulence). We also evaluated the local epidemiological situation and the globally spread lineage of ST2.
Results: The whole-genome analysis revealed that according to the Pasteur scheme, the majority of the isolates were globally disseminated clones of ST2 (n = 25), while less frequent sequence types included ST636 (n = 6), ST1 (n = 4), ST85 and ST78 (n = 2 each), and ST604, ST215, ST158, and ST10 (n = 1 each). Using the Oxford typing scheme, we identified two novel types (ST2454 and ST2455). While the majority (26/29) of blaOXA-23 genes were chromosomally carried, all blaOXA -72 genes were plasmid-borne. High heterogeneity in KL types and ST was observed. The modern clinical isolates differed from broadly used and historically established strains in colony morphology, cellular density, capsule production and virulence. Therefore, we propose a first comprehensive database called Acinetobase (https://acinetobase.vib.be/), which includes the bacterial strains and the associated phenotypic and genetic data.
Conclusion: Our results show the presence of high-risk clones of A. baumannii within Belgian healthcare facilities with frequent occurrences of genes encoding carbapenemases and therefore the need for constant surveillance. We have also established the first database providing complex information such as genomic, phenotypic data and the strains of Acinetobacter. This new implementable repository, freely accessible to the entire community, allows the selection of the best bacterial isolate(s) related to any biological question, using an efficient and fast exchange platform.

P03 | MALDI-TOF MS-based approach to uncover new Acinetobacter taxa in cattle feces

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Background: Acinetobacter is a taxonomically heterogeneous, ubiquitous genus with 76 correctly named species and many other provisional species. In contrast to the situation in humans, little is known about the diversity of Acinetobacter spp. in non-human ecosystems. Studies addressing this shortage could be complicated because of the involvement of samples with taxonomically convoluted compositions. The aim of this study was to develop and evaluate an efficient approach to assess the diversity of culturable Acinetobacter spp. Using cattle feces as a sample type.

Methods: Aliquots of homogenized feces were cultured aerobically with vigorous shaking in mineral medium supplemented with sodium acetate. Two growth temperatures, 30°C and 44°C, were applied in parallel for the same sample as they reflect different growth preferences of Acinetobacter spp. of human or environmental origin. Grown-up liquid cultures were streaked onto both mineral medium agar with acetate and CHROMagar™ Acinetobacter plates. Up to 24 agar-grown colonies per sample were directly identified by MALDI-TOF MS using the current Bruker database supplemented with the homemade entries of additional Acinetobacter taxa. Acinetobacter isolates were purified and reanalysed. Obtained spectra were then compared using cluster analysis to dereplicate isolates of a given species; final dereplication was done by macrorestriction analysis.

Results: A total of 500 Acinetobacter isolates were recovered from 28 samples collected in as many Czech farms in 2022, with 330 unique/dereplicated isolates being further studied. Among these, 14 correctly named species and at least 14 novel provisional taxa were identified. Using the growth temperature of 30°C, 70% of all bacterial isolates were identified as Acinetobacter and these belonged to all the species or taxa found in the study. In contrast, using 44°C, 42% of bacterial isolates were allocated to Acinetobacter, which belonged only to A. baumannii and two novel taxa. The recovery of A. baumannii was nevertheless more effective using 44°C as compared with 30°C.
Conclusions: We have developed an effective approach based on MALDI-TOF MS, which enables economical screening of multiple Acinetobacter isolates to assess their taxonomic diversity and to identify putative novel species.

P04 | Characterization of tigecycline (tet(X3)) and multidrug-resistant Acinetobacter lwoffii/pseudolwoffii from French animals

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Background: Tigecycline is a last-resort antibiotic restricted to humans for treating severe infections caused by multidrug-resistant bacteria. Plasmid-located tigecycline-resistance genes tet (X) have been characterized since 2019 in Enterobacterales and Acinetobacter species. The dissemination of tet(X) genes is favored by the ISCR2 insertion sequence and localization on transferable plasmids where they co-localize with other resistance determinants, such as blaNDM-1 and blaOXA-58. In the current study, the genetic content of animal Acinetobacter lwoffii/pseudolwoffii exhibiting tigecycline non-susceptibility was characterized.

Material and methods: During 2016-2022, four isolates presumptively identified as tigecycline nonsusceptible A. lwoffii (E-test 2 mg/L) were collected through the diagnostic laboratories of the Resapath (https://resapath.anses.fr). Genomes were sequenced by short (Illumina) and, for two isolates, by long reads (Oxford Nanopore) approaches. Assembly was performed using Shovill v.1.0.4 and Unicycler v.0.4.8. Identification was confirmed by rMLST (https://pubmlst.org/speciesid). Further bioinformatics analyses were conducted using the BC-BVR platform (https://www.bvbrc.org). Results: rMLST assigned two isolates to A. pseudolwoffii (43620 and 49430) and two to A. lwoffii (48934 and 61450) species. Isolate 48934 was from a cat, the remaining isolates from horses. All of them harbored a tet(X3) gene surrounded by a tyrosine site-specific recombinase and a resolvase encoding gene, in turn inserted on a ISCR2-like delimitated module located on non-typable plasmids with predicted structures for self-transfer. In isolate 43620, the tet(X3)-carrying plasmid harbored two multi-resistance regions, one harboring the blaVEB-1 extended spectrum betalactamase gene. Isolate 61450 presented a similar configuration. In isolate 49430, a blaOXA-58 gene was located on an additional plasmid (Figure 1). Conclusion: tet(X) genes and tigecycline resistance in Acinetobacter spp. were so far unreported among French animals. In general, tet(X) occurrence in Europe is rare, with two reports in tissues of dead animals from Italy and one from environmental source from Norway. However, their localization on predicted mobile elements could rapidly change the epidemiological picture.

Surveillance of this major resistance mechanism is important for animal and human health. Furthermore, these results underline the necessity of rationale usage of “old” antibiotics such as tetracycline, which regained interest to avoid the use of last-generations antibiotics but could coselect multiple and major resistance mechanisms in animals.

P05 | Proposal for Acinetobacter higginsii sp. nov. to accommodate organisms of human clinical origin previously classified as Acinetobacter genomic species 16

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Background: In 1989, Bouvet and Jeanjean delineated five novel proteolytic genomic species (GS) of the genus Acinetobacter, each with two to four human isolates. Three of these GS were later validly named, whereas the remaining two (GS15 and GS16) are still awaiting nomenclatural clarification. Here we present the results of a taxonomic study of 13 human strains classified as GS15 (n=3) or GS16 (n=10).
Methods: Four of the strains were studied by Bouvet and Jeanjean, while the additional nine were isolated from clinical specimens in the Czech Republic (n=7), Germany (n=1), and France (n=1) between 1955 and 2021. The 13 strains were recovered from urine, wound, sputum, lungs, liver abscess, or skin. Genus-wide comparison of the strains was carried out using metabolic and physiological testing, whole-cell MALDI-TOF MS profiling, and the analysis of whole-genome sequences determined by Illumina MiSeq sequencing.
Results: Based on whole-genome phylogenetic analysis, the strains formed two respective but closely related phylogroups within the so-called Acinetobacter hemolytic clade, with Acinetobacter dispersus as their closest neighbor. The intraspecies genomic ANIb values for GS16 and GS15 were 94.6–99.3% and 98.4–99.7%, respectively, whereas those between them reached 91.1– 93.1%. The ANIb values between the genomes of GS16 or GS15 and those of the known Acinetobacter species were ≤91.0%. All the 13 strains were non-glucose acidifying, strongly hemolytic and proteolytic, and grew at 37°C but not at 41°C. The two GS had highly similar catabolic profiles based on the utilization of diverse compounds as single sources of carbon and energy. Whole-cell MALDI-TOF MS-based cluster analysis differentiated both GS from other species but not from each other. Searching the NCBI database revealed genome sequences of additional GS16 strains isolated from humans, but none of GS15.
Conclusions: Our data support the status of GS16 as a novel species, but leave the question of the taxonomic status of GS15 open, given its close relatedness to GS16 and a limited number of available strains. We propose the name

Acinetobacter higginsii sp. nov. for GS16, with the type strain NIPH 1872T (= ATCC 17988T = CIP 70.18T = CCUG 996T = LMG 1031T).

P06 | Acinetobacter bovis sp. nov., a small-chromosome species found in cattle feces

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Background: We conducted a genus-wide taxonomic study of 15 strains of a putative novel Acinetobacter species isolated during a prospective study of the taxonomic diversity of acinetobacters in bovine feces from cattle farms in Czechia.

Methods: The 15 strains were isolated from bovine feces collected in eight Czech farms between 2021 and 2022 using enrichment technique in a minimal mineral medium supplemented with sodium acetate. The strains were compared with all known Acinetobacter species using standardized in-house metabolic and physiological testing, whole-cell MALDI-TOF mass spectrometry and the analysis of whole genomes determined by Illumina MiSeq sequencing. One strain was sequenced also with Nanopore MinION. The genomes were de novo assembled using Velvet v1.1.04 or Unicycler v0.5.0. Results: The draft whole-genome sequences of the strains were 2.5–2.6 Mb in size and had GC content of 36.3–36.7%. The complete genome of ANC 7201 consisted of a circular chromosome of 2,629,978 bp and four plasmids sized 51,375 bp, 11,751 bp, 4,795 bp, and 1,384 bp. Based on the phylogenetic analysis of the core genome, the 15 strains formed a distinct and internally coherent phylogroup within the genus, with Acinetobacter portensis being the closest species. The genomic ANIb values for the 15 strains ranged between 97.0 and 98.9%, whereas those between them and the type strains of the known Acinetobacter species were ≤86.2%. Whole-cell MALDI-TOF MSbased cluster analysis verified the taxonomic distinctness of the novel group. All the 15 strains were non-glucose acidifying, nonhemolytic, nonproteolytic and growing at 37 °C but not at 41 °C, and utilized acetate, L-aspartate, ethanol, and L-glutamate but not adipate and 2,3-butanediol as single sources of carbon and energy. This phenotypic profile distinguishes the novel strains from all known Acinetobacter species. Conclusions: We infer that the 15 strains represent a novel species associated with cattle feces, for which the name Acinetobacter bovis sp. nov. is proposed. The type strain is ANC 7201T (= CCM 9289T = CCUG 76665T); its genome consists of one of the smallest complete chromosome known for the genus.

P07 | Species distribution and antibiotic susceptibility of Acinetobacter isolates from non-hospitalized patients

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Background: Our knowledge about the occurrence of individual Acinetobacter spp. in humans is mainly based on studies of isolates from hospitalized patients, leaving open the question about the species' distribution in humans in non-hospital settings. To address this issue, we conducted a prospective taxonomic study of isolates recovered from non-hospitalized patients.

Methods: Clinical specimens were collected and acinetobacters isolated in a Czech regional laboratory servicing several ambulatory health-care facilities in 2014−2020. Species identification was done using a combination of whole-cell MALDI-TOF MS, rpoB gene sequencing, blaOXA-51like gene detection, and phenotyping. Subtyping was performed by macrorestriction analysis. Antimicrobial susceptibilities were determined by disk diffusion, resistance genes were detected by PCR.
Results: A total of 300 non-replicate isolates (one isolate of a given species per patient) were obtained from 298 patients, with urine (29%), skin defects (29%), and ear/nose/throat swabs (27%) as the most common specimens. As many as 290 (97%) isolates were identified to 22 validly named species, with A. pittii (n=108), A. baumannii (n=41), A. lwoffii (n=24), and A. ursingii (n=23) being most frequent. Each of the following species included <15 isolates: A. beijerinckii, A. bereziniae, A. calcoaceticus, A. courvalinii, A. dispersus, A. gyllenbergii, A. haemolyticus, A. indicus, A. johnsonii, A. junii, A. lactucae, A. modestus, A. nosocomialis, A. radioresistens, A. seifertii, A. schindleri, A. soli, and A. vivianii. Although the identification of the remaining 10 isolates was not conclusive, most of them were related to known species. Two hundred-ninety (97%) isolates were susceptible to (nearly) all antibiotics, which are primarily effective against wild-type susceptible A. baumannii. Exceptional were 10 multidrug-resistant A. baumannii from the skin defects of patients with a history of hospitalization. These isolates belonged to international clone 2, carried the blaOXA-23 and/or armA genes and shared macrorestriction patterns with strains endemic in the hospital.
Conclusions: Acinetobacters occurring in non-hospitalized patients appear taxonomically more diverse and susceptible to antibiotics compared to those from hospital settings. Notable is the predominance of A. pittii, probably the major human species in non-epidemic situations. Our study further confirms that the current classification of the genus sufficiently covers Acinetobacter spp. present in humans.

P08 | The classification of Acinetobacter species in the light of phylogenomics

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Background: In February 2022, the genus Acinetobacter included 76 species with correct, validly published names. However, available data, including whole-genome sequences deposited in the NCBI database, suggest that the real taxonomic diversity of the genus is more complex and far from being satisfactorily understood. Here we present an up-date of the phylogenomics-based reconstruction of the genus taxonomic structure in relation to its formal nomenclature.
Methods: Included in the study were the whole-genome sequences of the type/reference strains of all species with validly published names, species with effectively but not validly published names, and provisional species as well as taxonomically unique sequences available from NCBI. A phylogenetic tree was constructed based on concatenated groups of core orthologues; it was supplemented with the basic statistical characteristics of the sequences. Genome similarities were calculated using the average nucleotide identity (ANIb) and digital DNA–DNA hybridization (dDDH) parameters.
Results: The genomes were 2.4–5.0 Mb in size, with the GC content ranging from 34.9 to 49.6 mol %. The phylogram revealed a number of distinct phylogroups within the genus, with some associated with unique habitats, such as poplar bark or floral nectar. The two most notable phylogroups corresponded to the A. calcoaceticus-A. baumannii complex and the so-called hemolytic clade. Other examples are phylogroups each containing ecologically dissimilar species (A. baylyi and A. soli or A. bereziniae and A. guillouiae), a broad phylogroup associated with soil or water ecosystems (A. albensis, A. bohemicus, A. terrae, and A. terrestris), and a group encompassing catabolically versatile and ubiquitous species (A. lwoffii, A. pseudolwoffii, A. schindleri, and A. variabilis). The genus-wide phylogram together with ANIb/dDDH values revealed the synonymy of several validly and only effectively published names, indicated several potentially novel species, and identified taxonomically complex clusters of sequences with rather continuously distributed pairwise ANIb values of ≥91 (e.g. those typified by A. calcoaceticus or A. pittii).
Conclusions: Acinetobacter is a phylogenetically deep and genomically highly heterogeneous genus, which leaves open many challenging questions regarding its taxonomy and evolution, including questioning the technocratic application of quantitative taxonomic thresholds.

P09 | Frequency of Acinetobacter species isolated from clinical samples over a 34month period

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Background: The majority of published reports on clinical Acinetobacter spp. involve Acinetobacter baumannii, particularly multi-drug resistant isolates. We sought to determine the frequency of all Acinetobacter spp. that were cultured from clinical samples in our routine microbiology diagnostic laboratory between November 2019 and September 2022.

Methods: All clinical specimens submitted to the diagnostic laboratory were processed using standard laboratory methods. Species were identified using MALDI-TOF, and Acinetobacter baumannii group isolates were further identified using gyrB multiplex PCR. Whole genome sequencing (WGS) was performed on a MiSeq. Assembled genomes (Velvet) were submitted to JSpeciesWS to confirm species identification. One unique isolate per patient was retained for this study.
Results: From a total of 512 consecutively obtained Acinetobacter isolates collected from a wide variety of specimen types (Table 1), 493 were determined as unique and were isolated from; blood, n=3; abdomen, n=17; rectal swabs, n=46; urinary tract, n=119; respiratory, n=148; and wound and skin swabs, n=160. Thirty-eight patients had >1 Acinetobacter species, or >1 unique strain within a species. The most frequently isolated species were members of the A. baumannii group, in particular A. baumannii and A. pittii, with A. pittii the most frequently isolated Acinetobacter accounting for 43.2% of the total (Table 1). Other species such as A. bereziniae, A. johnsonii, A. junii, A. lwoffii, and A. ursingii were also found in >2% of isolates. Using the assembled genomes, we found that most of the A. dijkshoorniae (synonym of A. lactucae) were A. pittii. Similarly, five A. seifertii isolates were also found to be A. pittii. gyrB multiplex PCR correctly identified A. baumannii, A. calcoaceticus and A. nosocomialis. However, it did not differentiate between A. pittii and A. dijkshoorniae. Genomic data revealed several Acinetobacter species that were always misidentified by MALDI-TOF, although other species were correctly identified. Six isolates belonged to 6 unnamed Acinetobacter species.

Conclusions: The most common Acinetobacter sp. isolated in our hospital was A. pittii, followed by A. baumannii. MALDI-TOF was unable to correctly identify some Acinetobacter spp., especially A. pittii. We suggest using a molecular method to confirm Acinetobacter species identification.

P10 | Characterization of NDM-producing Acinetobacter bereziniae strains isolated in Chilean hospitals

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Background: Strikingly, the emergence of NDM-producing Acinetobacter spp. isolates has raised alarm. Accordingly, the Instituto de Salud Pública de Chile (ISPCh) decreed an alert due to the first description of NDM-producing Acinetobacter spp. isolates collected in 2020 in the country. In this work we characterized two strains of NDM-producing Acinetobacter spp. collected in Chilean hospitals between 2020 – 2021. Methods: UCO-553 isolate collected from tracheal aspirate and UCO-554 recovered from blood samples were analysed. Antibiotic-resistance profiles were determined for all strains by disk diffusion test and the presence of diverse carbapenemases was investigated by PCR. All isolates were subjected to whole-genome sequencing (WGS) using the Illumina (short-reads) and Nanopore platforms (long-reads). The genomes were assembled using a hybrid approach and annotated using Proksee and Bakta. The genomes were used for MLST and cgMLST analysis while resistance genes were detected using ResFinder. Moreover, conjugation experiments were carried out with an A. baumannii recipient strain. Results: UCO-553 and UCO -554 were identified as A. bereziniae, were STPAS1761, and cgMLST revealed that they differ in 19/3247 alleles, which is interpreted as not originating from the same source. Both isolates were resistant to cephalosporins, carbapenems, fluoroquinolones, tetracycline, cotrimoxazole, aminoglycosides and colistin. Both harboured the intrinsic blaOXA-229 gene. UCO-553 presented five plasmids ranging between 5.5 kb – 38 kb, whereas UCO-554 contained four plasmids ranging from 5.5 kb – 47 kb. The resistome of UCO-553 was composed by aph(3')-VI, aph(6)-Id, aph(3'')-Ib, ant(2'')-Ia, sul2, tet(Y) and blaNDM-1, whereas UCO-554 contained the aph(3')-VI, aph(6)-Id, aph (3'')-Ib, sul2, ant(2'')-Ia, tet(Y) and blaNDM-1 genes. Moreover, the blaNDM-1 gene was embedded in a Tn125 transposon in two different plasmids: 9-kb plasmid (UCO-553) and 47-kb plasmid (UCO -554). Interestingly, these plasmids were mobilised into A. baumannii by conjugation. Conclusion: We conclude that the Acinetobacter bereziniae isolates circulating in hospitals in Chile encoded a broad resistome, where the blaNDM-1 gene was located in different plasmids harbouring the Tn125 transposon, and the plasmids were mobilisable into A. baumannii, representing a challenge for surveillance in the hospital settings.

P11 | Phenotypic and genotypic characterization of Acinetobacter baumannii clinical isolates: yesterday and today

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Background: Acinetobacter baumannii has become notable as a cause of nosocomial infections. It showed remarkable genomic plasticity due to mutational variations and horizontal gene transfer to increase its fitness in hospital environment. Therefore, as a step forward in understanding its evolution under antibiotic selective pressure, this study aims to compare the resistome and virulome of A. baumannii clinical isolates collected from the last decade. The phenotypic properties of the A. baumannii clinical isolates will also be addressed. Methods: A total of 30 clinical A. baumannii isolates were collected from a public hospital in Rome during 2010-2023. Isolates were collected mainly from respiratory and urine specimens. Antibiotic susceptibility was determined by minimum inhibitory concentration according to EUCAST. Biofilm-forming ability was evaluated by crystal violet staining; motility and protease assays were also performed. The ability to adhere to human alveolar and bladder epithelial cells is ongoing. Results: The majority of the isolates were nonsusceptible to various agents of carbapenem, aminoglycoside, fluoroquinolones, β-lactamase, and folate pathway inhibitors, while less than 10% of strains were colistin-resistant. Preliminary results showed that 60% of strains isolated from respiratory infections displayed moderate to strong biofilm-forming activity compared to those from urinary sites. This ability allows bacteria to promote their lifespan on abiotic surfaces, i.e., ventilators, catheters, or surgical tools. Motility is involved in persisting in a stressed environment; only 10% of the isolates exhibited marked twitching motility on soft agar plates, 35% had some degree of motility, whereas the remaining 55% were not motile. All strains showed low protease activity on skim milk agar plates. Conclusions: Current work with whole-genome sequencing is ongoing; these analyses will provide the genomic profile associated with antimicrobial resistance, virulence factors, multi-locus sequence types, and the relationship among isolates. Characterizing their chromosomal and plasmid-encoded resistance and virulence traits could help us understand the mechanisms behind the genetic mobilization and spread of these genes among these opportunistic pathogens and clues about their evolution as a response to environmental stress conditions. This comparative study could spotlight valuable information on preventing transmission in a healthcare environment.

P12 | Acinetobacter baumannii in a primary hospital from Portugal: distribution and antibiotic susceptibility patterns during five years (2018-2023)

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Introduction: Acinetobacter baumannii is an opportunistic gram-negative pathogen, recognized for causing a wide variety of infections, namely nosocomial infections. This has become a difficult to treat pathogen worldwide due to its ability to acquire multi-drug resistance, particularly to carbapenems and aminoglycosides.
Methods: In this study, we investigated the distribution of Acinetobacter baumannii isolates taking into account several factors such as the type of sample, inpatients service, demographic information. The pattern Of Antibiotics susceptibility was also analyzed.

From January 2018 to December 2023, we collected the microbiology laboratory data of Acinetobacter baumannii isolated from patients hospitalized at a primary hospital in Portugal. Identification and antibiotic susceptibility test were done by MicroScan WalkAway ID/AST system (Beckman Coulter).

Results:
A total of 68 isolates of Acinetobacter baumannii were included. Urine accounted for the majority of specimens, followed by abdominal fluids and respiratory samples (33,8%, 22%, 20,6% respectively). The patients had a median age of 69 years (min. 4; max. 94), mostly male (n=43). The services with the highest number of isolations were surgery (22%), intensive care (17,6%) and urgency (20,6%). The incidence of all species did not change significantly according to the years, being the maximum reached in the year 2020 (n=19).

Conclusions: It is concluded that the number of isolations as well as their resistance profile do not seem to be increasing or getting worse over the analyzed period. However, in the future, it would be important carry out more studies in order to assess the clinical implications and therapeutic management in patients with A. baumannii infections.

P13 | Isolation and characterisation by whole genome sequencing of Acinetobacter spp. collected from raw meat and meat products in Spain

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Background: Acinetobacter spp. is an important nosocomial pathogen related to antibiotic resistant infections. Although raw meat is a reservoir and vector of multidrug-resistant bacteria and antimicrobial resistance genes (ARG), there is scarce information about the risk of spread of antimicrobial resistant Acinetobacter spp. Through the meat production chain.

Methods: A total of one hundred samples of meat and meat products (pork, beef, chicken, turkey and mixed meat products), both unpackaged, vacuum packed or packed under protective atmospheres were analysed. The selective medium CHROMagar™ Acinetobacter was used for detection and enumeration of Acinetobacter spp., and the same medium with MDR Selective Supplement CR102 to isolate carbapenem-resistant Acinetobacter strains. The presumptive Acinetobacter isolates were identified by MALDI-TOF mass spectrometry. Moreover, a total of 129 Acinetobacter spp. isolates were whole genome sequenced by Illumina 150bp PE. Raw reads were processed by Trimgalore and assembled with SPAdes. Taxonomic assignment and antimicrobial resistance genes and plasmid detection were performed with GTDB-Tk, Blastn versus the ResFinder database, and platon software, respectively. Results: Acinetobacter spp. was isolated from 74 out of 100 samples, in concentrations of 1-100 CFU/g, however, none of the samples were positive for carbapenem-resistant Acinetobacter spp. A. baumannii and A. guillouiae were the most frequently isolated species with a 37% and 30% prevalence, respectively. Among the 129 sequenced strains, 107 carried at least one ARG. The most frequent ARGs detected were blaADC-25 (52%), tet(39) (22%) , blaOXA-274 (14%) and blaOXA-275 (14%). As relevant results, one mcr-4.3 gene and one blaOXA-58, both harboured in plasmids, were identified in an A. baumannii and an A. gerneri strain, respectively.

Conclusions: Raw meat and meat products are an important reservoir of different species of Acinetobacter, and particularly of A. baumannii, nevertheless the risk of exposure to carbapenemresistant Acinetobacter spp. through the consumption of meat products appears to be low.

P14 | Acinetobacter abundance in cattle feces from Czech farms

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Background: Fresh animal feces and farm manure seem to be a reservoir of various Acinetobacter spp., but detailed studies on factors conditioning the presence and abundance of acinetobacters in such environments are missing. Here, we assessed Acinetobacter abundance in cattle feces in relation to the in-farm antibiotic use and other farm- and cow-specific factors. Methods: Cattle feces were sampled in 28 Czech farms (both dairy and beef) with contrasting antibiotic use. Either fresh fecal samples were taken from individual cows (from rectum or at the moment of defecation), or mixed fecal samples (~several hours old) were collected on the farm floor. Total DNA was extracted from feces using DNeasy PowerSoil kit and the abundance of Acinetobacter was measured using qPCR with Acinetobacter-genus specific primers, targeting the 16S rRNA genes.

Results: In fresh feces from individual cows (n=94), the maximum absolute abundance of Acinetobacter was 5.69E+09 16S rRNA copies/g dry weight, corresponding to approx. 2% of total bacteria. About half of the fresh fecal samples remained below the limit of detection (~ 1E+06 copies/g dry weight). In mixed fecal samples from farm floor (n=28), the maximum and average abundance of Acinetobacter were 3.47E+10 and 4.33E+09 16S copies/g dry weight (7 and 1% of total bacteria), respectively. Statistical comparisons showed significantly higher Acinetobacter abundance in floor samples when compared to per-farm mean values from individual cows. Further comparisons of Acinetobacter abundance between bio vs. conventional farms, dairy vs. beef farms, and indoor vs. outdoor stabling did not reveal any significant differences. Correlation tests did not indicate any relationship between Acinetobacter abundance and on-farm antibiotic use, size of herd, cow age, sample pH, and sampling temperature. Conclusions: Acinetobacters seem to proliferate in feces deposited on farm floor, perhaps due to their higher competitiveness at lower temperatures and higher oxygen levels, as compared to the intestine conditions. Other underlying factors behind Acinetobacter abundance in cattle feces remain unknown. Further analyses of chemical composition of cattle feces as other explanatory factors are ongoing.

P15 | Hi-GRIL-seq uncovers a posttranscriptional regulator of CarO and BfnH in Acinetobacter baumannii AB5075

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Acinetobacter baumannii is an opportunistic, Gram-negative pathogen that causes nosocomial infections in critically-ill hospitalised patients. This organism is a leading source of antimicrobial resistant infections, prompting the World Health Organisation to recognise A. baumannii as a priority pathogen. A. baumannii thrives in clinical settings due to its ability to rapidly alter expression of critical survival genes. While several small RNA (sRNA) candidates were discovered, no study has mechanistically characterised antisense base-pairing interactions with mRNA molecules in this species, yet. In order to identify mRNA targets for each candidate sRNA, we performed the proximity ligation protocol High throughput Global sRNA target identification by Ligation and Sequencing (Hi-GRIL-seq), which led to the identification of sRNA-mRNA interactions in A. baumannii AB5075. We experimentally validated that the sRNA RcbS interacts with the mRNAs of the outer membrane protein CarO, the siderophore receptor BfnH, a drug-metabolite transporter and neutral zinc metallopeptidase in vitro and in vivo. We found that RcbS negatively regulates expression of carO and bfnH mRNA transcripts through the use of a conserved "seed" sequences that mediates RcbS-mRNA annealing using in-line probing. We then validated the RcbS-carO mRNA interactions in A. baumannii. This study demonstrates that Hi-GRIL-seq enables identification of novel sRNA-mRNA interactions in a Hfq-independent manner. The biological function of RcbS remains under investigation, however, we found that this sRNA is upregulated in various stress conditions suggesting that it may play a role in envelope stress responses in this pathogen. We provide novel insight into posttranscriptional regulation in A. baumannii. Our HiGRIL-seq database may be used to functionally characterise both known and unknown A. baumannii candidate sRNAs and may provide further insight into post-transcriptional regulation of A. baumannii.

P16 | Transcriptomic analysis of A. baumannii AB5075 with (differential)-RNA sequencing

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Acinetobacter baumannii is a major threat to human health because it is an opportunistic, multidrug resistant nosocomial bacterium. A. baumannii has become endemic in hospitals due to its diverse genetic machinery, which allows it to rapidly generate resistance factors, and its extraordinary ability to withstand harsh environments. Because of its clinical relevance as a multidrug resistant (MDR) bacterium at the top of the World Health Organization's (WHO) list of priority pathogens that urgently require research, discovery, and development of new antibiotics, A. baumannii is the ideal model organism for studying and dissecting how antimicrobial resistance genes (ARGs) are regulated. However, an underutilised but potentially very effective strategy for combating AMR is to study when and how bacteria switch between sensitive and resistant phenotypes by regulating the expression of ARGs. In this study, I aim at assessing the contribution of two-component system (TCS) to antibiotic resistance and the primary transcriptome of A. baumannii AB5075. As a starting point, I have generated a clean baeR mutant of which RNA was isolated and sent for RNA sequencing. Antibiotic resistance and susceptibility tests of WT and ΔbaeR strains were carried out, and the WT and ΔbaeR so far do not show any differences. In parallel, I defined the location of 5955 transcriptional start sites (TSS) in A. baumannii AB5075 by dRNA-seq. This research will advance knowledge of the genes controlling and causing antibiotic resistance in the AB5075 strain and define the location of the promoters that drive their expression.

P17 | Characterization of a genome database of 837 isolates assigned to 72 distinct species in the Acinetobacter genus using the Pasteur Multi-locus sequencing type (MLST) scheme.

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Background

Non-baumannii Acinetobacter species are increasingly isolated in the clinical setting and the environment. The aim of the present study was to analyze a genome database of 837 Acinetobacter spp. isolates, which includes 798 non-baumannii Acinetobacter genomes, in order to define concordance of classification and discriminatory power of 7-gene MLST, 53-gene MLST and single-nucleotide polymorphisms SNPs phylogenies.

Methods

Bacterial genomes were manually selected from the PubMLST database. Pasteur MLST and ribosomal MLST were performed using BIGSdb software. Phylogenies were performed on Pasteur MLST or ribosomal MLST concatenated alleles, or SNPs extracted from core genome alignment using RAxML under the GTR-GAMMA model. The phylogenetic trees were visualized using iTol v6 software. The resistance genes were detected using ABRicate software.

Results

The Pasteur MLST scheme was able to identify and genotype 72 species in the Acinetobacter genus, with classification results concordant with the ribosomal MLST scheme. The discriminatory power and the genotyping reliability of Pasteur MLST scheme was assessed in comparison to genome-wide SNP phylogeny on 535 non-baumannii Acinetobacter genomes assigned to Acinetobacter pittii, Acinetobacter nosocomialis, Acinetobacter seifertii and Acinetobacter lactucae (heterotypic synonym of Acinetobacter dijkshoorniae), which are the most clinically relevant nonbaumannii species of the A. baumannii group. The Pasteur MLST and SNP phylogenies were congruent (Robinson-Fould test) and grouped genomes into four monophyletic and six nonmonophyletic clades in A. pittii, and one each in A. seifertii, respectively. Also, A. lactucae genomes were grouped into one non-monophyletic clade within A. pittii genomes. The SNP phylogeny of A. nosocomialis genomes showed a heterogeneous population and did not correspond to Pasteur MLST phylogeny, which identified two monophyletic and three non-monophyletic clades. Acquired antimicrobial resistance genes belonging to at least three different antimicrobial classes were identified in 122 isolates assigned to 21 distinct species in the Acinetobacter genus. Also, the presence of a class D oxacillinase, which is a naturally occurring enzyme in several Acinetobacter species, was found in 482 isolates assigned to 35 Acinetobacter species.

Conclusions

The Pasteur MLST scheme is a useful genotyping tool to identify Acinetobacter species and to analyze the population structure of the A. baumannii group.

P18 | Identification of the minimal region involved in the replication of a widespread Acinetobacter plasmid family carrying an NDM-1 gene

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Background

The New Delhi Metallo-beta-lactamase blaNDM-1 and its variants confer resistance to all β-lactam antibiotics, except monobactams and the amidinopencillin, mecillinam. In recent years, the blaNDM -1 gene and its derivatives have spread rapidly, limiting the therapeutic options of patients infected with Multi-Drug Resistant microorganisms. These genes can be found in many members of the Enterobacteriaceae family but also in Pseudomonas aeruginosa or Acinetobacter baumannii. The blaNDM genes are linked to a wide variety of plasmids, including IncC, IncFII, IncFIB, IncH, IncL/M, IncX3, or to untyped plasmids, but always in the vicinity of transposons. Recently, two large Acinetobacter plasmid clades carrying the blaNDM genes were described. One of them group plasmids encoding a Rep protein of the Rep_3 superfamily. The other one (pNDMYR7 clade) embrace conjugative plasmids of medium size, but the gene encoding the replication initiation gene (Rep) remained, until now, unrecognized. Plasmid pAhaeAN54e is an Acinetobacter haemolyticus medium-size replicon (45.46 Kb), identified in strain AN54 isolated in a Mexican hospital, containing an NDM-1 gene. This plasmid belongs to the pNDM-YR7 clade and their Rep protein was not recognizable by bioinformatic means. Here we present the identification of the minimal region of plasmid pAhaeAN54e involved in plasmi replication.

Methods

Regions containing genes annotated as hypothetical of plasmid pAhaeAN54e or large regions without annotated CDS were cloned in a mobilizable suicide vector and transferred to an AN54 derivative lacking pAhaeAN54e.

Results

Eight regions containing genes encoding hypothetical proteins or regions without annotated CDS were amplified by PCR and cloned into a mobilizable suicide vector. Recombinant plasmids were conjugated into an AN54 derivative lacking plasmid pAhaeAN54e. Only one construct, pF8, could sustain the replication of the recombinant plasmid in the AN54 cured derivative. This construct has an insert size of 1704 bp. Then, different DNA segments of the insert of pF8 were tested to determine their ability to replicate into the cured derivative. The minimal region required for replication has 834 pb. A BLASTn search showed that this region is also present in the plasmids of the clade pNDM-YR7 and in plasmids of other Acinetobacter species

P19 | Acinetobacter baumannii bacteraemia: clinical aspects and molecular characterization

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Acinetobacter baumannii bacteraemia remain rare but occur preferentially in catheterized, immunocompromised and long-term hospitalized patients. Given the large number of infections due to this bacteria in our hospital, it seems ideal to determine with statistically representative number the frequency of A.baumannii bacteraemia, their clinical aspects and the molecular characterization of the involved strains.

All cases of bacteraemia due to A.baumannii occur during three years (2015-2017) at the CHU Mustapha in Algiers were included. Clinical characteristics of patients such as age, reason of hospitalization, mortality rate were analysed. Each strain was phenotypically characterized by antibiogram according to CLSI criteria. The MICs for tigecyclin and colistin were determined respectively by E-test® and broth microdilution for carbapenem resistant strains. Among these, each strain was genotypically characterized by PCR for OXA-23, OXA-24, OXA-58, OXA-51, NDM, VIM, IMP and armA methylase. During these three years, 154 invasive forms of A.baumannii infections have been observed with 66 bacteraemia (43%), post-surgical deep suppuration infections 61 (40%), meningitis 12 (8%), pleurisy 8 (5%) and ascetic fluid infection 7(4%). We distinguish among bacteraemia those occurring in the paediatric population (group A) from those occurring in adults (group B). Group A (N=37): 26 bacteraemia occurred on neonatal ICU, six on pediatric oncology, three on pediatric surgery and two on neurosurgery. The mortality rate was 13,5% (5/37). The rate of carbapenem resistance was 17/37 (46%) and strains expressed OXA-23+ armA for 14 of them while the other three were NDM(+). Group B (N=29): 18 bacteraemia occurred on ICU, four on cardiac surgery, four on general surgery and three on neurosurgery. The mortality rate was 31% (9/29). The rate of carbapenem resistance was 24/29 (83%) and strains expressed OXA-23+ armA for 19 of them while the other five were NDM(+). Through this series, we demonstrate that in a country with a high prevalence of A.baumannii infections, bacteremia occurs preferentially in the pediatric population with, however, a significantly lower mortality rate than those occurring in adults, correlated with a rate of resistance to carbapenems also less important.

P20 | Ancestral state reconstruction of multi-drug resistant Acinetobacter species.

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Background: Acinetobacter baumannii can swiftly acquire antimicrobial resistance (AMR) and is responsible for most worldwide Acinetobacter infections, making it of critical interest. Focusing on this species enables us to understand the evolutionary pathways that enable it, and possibly other Acinetobacter species, to be increasingly associated with antibiotic resistance and human infections. Previous work has shown that several members of the Acinetobacter genus possess a large pool of encoded blaOXA β-lactamases. Consequently, we aimed at examining the mechanisms/rate of AMR gene acquisition and transmission in a large population of clinical Acinetobacter samples.

Methods: We investigated a total of 1259 Acinetobacter sp. Clinical genomes collected, between 1997 and 2021, primarily from countries in the Middle East and the Arabian Peninsula. Genomes were mined for AMR genes through the Comprehensive Antibiotic Resistance and the BetaLactamase databases. We performed ancestral character reconstruction of AMR gene presence/absence using PastML, on a maximum likelihood core genome phylogeny inferred with IQ-TREE.

Results: Geographically, samples were mostly distributed between Israel (586/1259; 47%) and Lebanon (506/1259; 40%). The most common β-lactamase gene was blaOXA-23, present in 691/1259 (55%) of genomes. The gene was acquired multiple times independently by different A. baumannii lineages, indicating frequent horizontal gene transfer. The gene is more commonly present in genomes further from the root, suggesting its increasing prevalence over time. There is clear geographic structure in the phylogeny, with most recently derived lineages corresponding to a single country. However, international transmission events are evident deeper in the tree, including a clade of mostly blaOXA-23 positive genomes which disseminated across multiple countries.

Conclusion: Our initial results highlight the importance of understanding the evolution of Acinetobacter species through the lens of AMR gene transmission. Our ongoing analysis will target additional AMR genes and include prophage regions for an in-depth understanding of events that shape Acinetobacter into resistant pathogens.

P21 | Transposition of ISAba13 in AB5075

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The clinical isolate of A. baumannii AB5075 harbours two copies of the IS5 family insertion sequence ISAba13. Here we show that ISAba13 can transpose into the capsule locus. RNA-seq reveals premature termination of transcription of this operon, and reduced expression of genes involved in synthesising the capsule. This causes a variety of phenotypic changes, including antibiotic susceptibility, biofilm formation, and natural competence. Transposition into this location has far reaching transcriptional effects beyond nearby genes, including downregulation of the major subunit of the type IV pilus, pilA. To investigate the potential genetic diversity that can be generated, we used IS-seq to natively map over 20,000 novel insertion sites of ISAba13 across the chromosome. Insertion sites show an unexpected propensity for AT-rich regions. In many gram-negative bacteria, such regions are bound by the global transcriptional regulator histone-like nucleoid-structuring protein (H-NS). Strikingly, in an hns mutant, this preference for transposition into AT-rich regions is abolished. This suggests H-NS directs transposition events, potentially via a DNA bridging mechanism.

These findings highlight the potential to generate huge genetic and phenotypic heterogeneity within bacterial populations. Additionally, they highlight the pivotal role that chromosome folding proteins play in transposition.

P22 | Genomic epidemiology and evolution of globally distributed carbapenem resistant Acinetobacter baumannii

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Antimicrobial resistant (AMR) Acinetobacter baumannii are priority pathogens for the World Health Organisation but little is known about their global epidemiology. Here we analyse a global dataset of A. baumannii genomes, including clinical isolates sampled over 16 years across Russia. Comparative genomic analyses characterized the rise and spread of resistant lineages and this was compared to AMR determined in vitro for eight clinically relevant antibiotics and in silico using publicly available databases. Next, genome-wide association studies (GWAS) were used to identify the genetic elements underlying carbapenem resistance and a phylodynamics approach inferred the timescale for emergence of AMR. Our bioinformatic analyses revealed a diverse pathogen population, including internationally disseminated clones and emerging lineages. A total of 83.3% (70/84) of carbapenem (Imipenem and Meropenem) resistant isolates belonged to just three lineages, including globally distributed Sequence Types (STs) 944, 231, 208 and their derivatives (ST-Oxford scheme). Our findings clearly illustrate an increasing trend in AMR A. baumannii infections and rapid emergence of resistance against the last generation carbapenem. As we begin to understand the relationship between antibiotic usage and AMR emergence, we can model AMR transmission networks for appropriate intervention strategies against this immediate public health threat.

P23 | The Acinetobacter baumannii genome and sequence reference libraries hosted on PubMLST.org

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The PubMLST Acinetobacter baumannii database has hosted allelic diversity data for multi-locus sequence typing (MLST) since 2010 and currently has records for approximately 8,000 isolates sampled from over 100 countries. The database began hosting genomic data in 2012. The database hosts assembled whole genome data for reference strains and increasingly for submitted isolates from across the world reflecting genomic diversity among A. baumanii. Isolate records are linked to publications and can be structured into coherent projects. It includes assembled genomes for 3,243 global isolates from across the genus Acinetobacter. Loci have been defined within the database for the core genome and parts of the accessory genome in a manner analogous to MLST so that sequence diversity is now indexed at >3,000 loci, with each unique gene sequence assigned an allele number. These loci are organised into schemes, including a core genome MLST (cgMLST) scheme for A. baumanii that facilitates clustering and identification of nearest neighbour genomes, and schemes for identifying emergent and outbreak strains. A range of analyses can be performed using built-in tools for comparative genomics. We have recently introduced customisable front-end dashboards that allow users to show graphical breakdowns of any fields of interest. A data explorer tool linked from dashboard elements allows inter-relationships with other fields to be investigated, with links that lead directly to datasets filtered by the selected field values.

The underlying platform, BIGSdb, is under constant development and recently introduced functionality and improvements facilitating whole genome analysis, clustering and visualisation will be presented.

P24 | Role of the competence protein ComC of Acinetobacter baumannii in natural transformation, twitching motility and adhesion

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Background: The human pathogen Acinetobacter baumannii is a leading cause of nosocomial infections worldwide. Several strains are naturally transformable and this trait is suggested to contribute to adaptation to the human host and increasing antibiotic resistances. Natural transformation is linked to migration in the solid medium-plastic interphase referred to as twitching motility mediated by type IV pili (T4P). Macromolecular DNA-transporter linked to T4P are suggested to mediate DNA uptake [1]. One potential DNA transporter protein, designated ComC, is broadly distributed in Acinetobacter species but exhibits significant differences in protein domain architecture in pathogenic Acinetobacter species such as containing a van Willebrand Factor type A domain (VWA). We addressed the role of ComC and the VWA domain in natural transformation, twitching motility and adhesion of A. baumannii AYE.
Methods: Natural transformation, twitching motility, adhesion, electron microscopy
Results: Genome screening of A. baumannii AYE led to the identification of a gene cluster encoding FimU, PilV, ComB, PilX, ComE, ComF and the potential tip adhesin ComC. A ΔcomC mutant was completely defect in transformation and twitching motility and displayed a significantely reduced adhesion rate to HUVECs. Complementation of the ΔcomC mutant with comC restored natural transformation, twitching phenotype and adhesion. Complementation of the ΔcomC mutant with comC lacking the VWA domain also restored the natural transformation phenotype but not completely. In contrast twitching motility and adhesion were not restored. No difference in piliation between the wild type and ΔcomC mutant was detected.
Conclusions: ComC of A. baumannii plays an essential role in natural transformation, twitching motility and adhesion. The VWA domain plays a crucial role in twitching motility and adhesion but is dispensable for natural transformation. This together with the abundance of the VWA domain in ComC of pathogenic Acinetobacter species suggests that the VWA domain plays an important role in ComC-mediated interaction of pathogenic Acinetobacter species with the human host.

[1] Averhoff B., Kirchner L., Pfefferle K., Yaman D. Natural transformation in Gram-negative bacteria thriving in extreme environments: from genes and genomes to proteins, structures and regulation. Extremophiles 25 (2021), 425-436.

P25 | The VBNC state as global stress response in different Acinetobacter baumannii strains

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Background: Apart from increasing multidrug resistances, the viable but non-culturable (VBNC) state is an additional challenge for the treatment of pathogens. This state is a stress-induced persistence state found in several Gram-negative bacteria. Under VBNC-conditions, bacteria become unculturable in medium which would normally support their growth. VBNC-cells are defined as metabolically active and more resistant to antibiotics but unable to grow. Here, we describe that A. baumannii can enter a VBNC state, a previously unrecognized feature of this pathogen.

Methods: Long-term survival, resuscitation analysis, flow cytometry measurements, electron microscopy. Results: Long-term survival studies revealed that several strains of A. baumannii lost culturability in extended stationary growth phases. Loss of culturability was enhanced in the presence of stressors such as high salt, anoxic conditions, cold stress, heat stress and desiccation stress. A combination of stressors even accelerated this process. However, when cells that had lost culturability were diluted in sterile phosphate buffered saline to remove the stressor and were incubated for two days, culturability could be regained, i.e. cells were resuscitated. To prove that cells were viable in the state of non-culturability, flow cytometry measurements with the fluorescent dyes Syto 9 (stains viable cells) and propidium iodide (stains dead cells) were performed. This live-dead-staining indeed identified these cells to be viable proving the entry into a VBNC state. Morphology of VBNC cells were further characterized by electron microscopy. Conclusion: These data demonstrate that various strains of A. baumannii are able to survive unfavorable environmental conditions by entering the VBNC state.

P26 | Within-host microevolution of multidrug resistant Acinetobacter baumannii

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Background
Acinetobacter baumannii is responsible for a wide range of infections including urinary tract infections (UTI). Little is known about genomic changes during colonization without antibiotic pressure and thereby host-driven evolution. Here we use whole-genome sequencing and phenotype assays to investigate within-host microevolution during a subclinical long-term UTI.

Methods
Lyon School of Veterinary medicine (France) gave us access to several A. baumannii strains isolated from a cat with UTI over a five-year period. The genome of each isolate was whole sequenced using long read (Nanopore Technology) and short read sequencing (Illumina). The genomes were assembled (Unicycler) and annotated (Prokka). Single nucleotide Polymorphisms (SNP) and gaps of the late isolates, in comparison to the first one, were called using SNIPPY and MAUVE respectively. The most variable genes and the functional categories to which they belong have been identified and the associated phenotypes (antimicrobial susceptibility, adhesion, biofilm and metabolism) have been compared among the isolates.
Results
The strains isolated from this five-year infection belong to the sequence type ST25 frequently associated with mammalian. The infection is surprisingly monophyletic with very few variations in genome sizes and nucleotide sequences. More than 80% of the SNPs are located in genic regions and are non-synonymous. Genes that are affected by SNPs, insertion or deletion are involved in adhesion, biofilm formation, cell division, antibiotic resistance and metabolism.

Despite that adhesion and biofilm genes were affected, all the isolates produce almost the same amount of biofilm on abiotic surfaces. Antibiotic resistance decreases during the course of infection probably in order to give way to other survival strategies such as metabolic specialization. Metabolic assays suggest that the strains adapt to use a wider variety of carbon sources.

Conclusions
We hypothesize that adhesion and biofilm formation genes accumulate variations during the infection because they encode adhesive structures at the surface of the cells such as pili which are highly immunogenic, and modification of these structures could lead to host immune escaping. As there is no antibiotic treatment during infection, resistance mechanisms are lost in favor of a more flexible metabolism more adapted to the urine.

P27 | Acinetobacter baumannii outer-membrane vesicles immunomodulate human pulmonary epithelial cells: Effects of antimicrobial resistance

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Introduction: Acinetobacter baumannii (Ab) is responsible for hospital-acquired and ventilatorassociated pneumonia, which is often caused by multidrug resistant strains. Ab has been found to release Outer Membrane Vesicles (OMV) that can elicit immune signaling in host macrophages and dendritic cells (1,2). Little is known of the effects of Ab OMV on pulmonary epithelial cells, another key contributor to host immune surveillance. Moreover, whether and how antimicrobial resistance may alter these effects remains obscure. The aim of this project was to characterize and compare immune responses by lung epithelial cells exposed to Ab OMV from ciprofloxacin susceptible (OMVs) and ciprofloxacin resistant (OMVr) strains. Methodology: Two Ab strains, one ciprofloxacin-susceptible and ciprofloxacin-resistant were used. MICs were determined by microdilution assay. OMV were isolated using the Exoeasy assay (Quiagen) kit after 4 h growth. OMV were characterized by Nanosight NS300 and Transmission Electron Microscopy. Protein concentration was determined by micro-BCA assay kit. Calu-3 human pulmonary epithelial cells were grown in Dulbecco's Modified Eagle Medium with 10 % Fetal Bovine Serum and 1% of penicillin and streptomycin solution until confluence and exposed to 15 µg/mL of OMVs and OMVr protein for 22 hours. Levels of immune mediators from supernatant were analyzed by Multiplex ELISA. Results: MICs for the strains were 1mg/mL (susceptible) and 512 mg/L (resistant). OMV from both strains produced classical membrane-contained OMV. OMV size and numbers were not different between both strains, however protein contents were lower in OMVr when compared with OMVs. OMVr, but not OMVs, significantly reduced IL1-Ra. OMV from either strain did not change levels of other immune mediators (IL-8, IL-6, IL-1beta, TNFalpha, GM-CSF, MCP-1, IL-12p40, IL-13, IL-10, IFNgamma, or IL-2). Conclusion: Ab OMV from a ciprofloxacin-resistant strain are not different from those released by a susceptible strain in size or numbers, however protein content was lower in OMVr. OMVs and OMVr may have different immune-modulatory effects on IL-1Ra synthesis. Further research now needs to characterize the final biological outcome of the immunomodulating effects of Ab OMVs in pulmonary epithelial cells.

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P28 | First molecular characterisation of colistin and carbapenem-resistant clinical isolate of Acinetobacter baumannii from Bosnia and Herzegovina

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Background:
According to the World Health Organization, bacterium Acinetobacter baumannii is the first on the critical priority list of pathogens in urgent need for new antibiotics. Objective of this study was to characterise first colistin and carbapenem-resistant Acinetobacter baumannii isolate recently emerging in hospital settings.
Methods:
A. baumannii isolate was collected from wound hospitalised patient under colistin treatment in UCH Mostar in Bosnia and Herzegovina. The isolate was identified with routine bacteriological techniques and confirmed by MALDI-TOF MS (Microflex LT mass spectrometer and MALDI Biotyper 3.0 software, Bruker Daltonics, Germany) on cell extracts. Antibiotic susceptibility was determined and interpreted according to the EUCAST. To study the mechanism of carbapenem resistance we relied on a qualitative genotypic diagnostic Eazyplex®SuperBug Acinetotest system (Amplex Biosystems GmbH, Giessen, Germany), detecting carbapenemase OXA-23, OXA-40, OXA -58, and NDM. For multilocus sequence typing (MLST) we relied on the Oxford MLST scheme with seven housekeeping genes (gltA, gyrB, gdhB, recA, cpn60, gpi, and rpoD). Fragments amplified by polymerase chain reaction (PCR; ProFlex™ 96-Well PCR System, Applied Biosystems) were sequenced (commercial service GENEWIZ, Azenta Life Sciences, Germany) and edited using the Geneious Prime software. Allele sequences and profiles retrieved from the A. baumannii MLST website were used to determine the sequence type (ST).
Results:
The isolate belongs to the ST231 of IC1 and has turned out to be highly resistant to colistin (MIC≥16 mg/L) and carbapenems (with MIC≥32 mg/L to both imipenem and meropenem). PCR confirmed that isolate carried blaOXA-40-like gene. Isolate has point mutations in pmrCAB operon genes, P170L point mutation in the pmrB gene and the R125H point mutation in the pmrC gene.
Conclusion:
Our results provide the first investigation on mechanisms of colistin resistance in the clinical isolates of A. baumannii un-UCH Mostar. Colistin resistance in A. baumannii in hospitalised patient receiving colistin treatment is the result of chromosomal mutations.

P29 | Misidentification among Acinetobacter spp. species other than A.baumannii with analysis of their resistance profiles and acquired resistance genes.

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Background
MALDI-TOF MS is becoming the main technique for rapid bacterial identification at species level in routine diagnostics. In Poland, not all diagnostic laboratories can use it and still many species are diagnosed only to the level of Acinetobacter calcoaceticus-Acinetobacter baumannii (Acb) complex or species other than A.baumanni are often misidentified. Although Acinetobacter baumanii (AB) is the most commonly isolated species, non-AB species such as A.lwoffii, A.pittii, A.nosocomialis or A. junii have also been identified as important in nosocomial infections and are becoming more common and therefore should be precisely described.
Methods
Isolates non-AB and Acb complex were identified by MALDI-ToF (matrix-assisted-laserdesorption/ionization-time of flight). Species affiliation was confirmed by sequencing of the RNA βpolymerase (rpoB) subunit. Sequences were compared to reference sequences using the BLAST tool in the NCBI database. Antibiotic susceptibility was determined based on the results of the MIDITECH-Analyser automated system. The most prevalent carbapenamase resistance genes were detected in multiplex PCR and RealTime-PCR.

Results
Among the 12 Abc isolates, the MALDI-Tof method confirmed 11 AB and 1 A.pittii. In addition, 38 different non-ABs were collected and identified. Among all isolates, 10.3% caused BSI, 15.4% PNEU, 41.0% SSTI and 33.3% UTI. Comparative sequence analysis of the rpoB gene indicated isolates of A.ursingii(n=11), A.pittii(n=8), A.lowfii(n=7), A.junii(n=6), A.calcolaeticus(n=3), A. genomospecies(n=2), A.variabilis(n=1) and A.indicus(n=1). In 25.64% of cases, the MALDI-ToF identification result was different from the result based on the rpoB gene sequence. The isolates were resistance to cefoperazone/sulbactam 76.9% and ciprofloxacin 61.5%. Single genes were identified as blaVIM(n=2), blaOXA-23(n=1), blaOXA-40(n=2), blaOXA-58(n=1), blaGIM(n=1), and blaOXA-66(n=1).

Conclusions
Species of the genus Acinetobacter excluded AB are difficult to identify, methods based on culture and biochemical tests are time-consuming and subject to considerable error. The MALDI-ToF technique allows for the identification of bacteria in a short time and with high accuracy. In the case of non-AB species, there are differences between MALDI-ToF identification and sequencing-based reference methods. Proper identification of non-AB species is very important, given their increasing spread and possibility of acquiring drug resistance. Future research should focus on better understanding non-AB species anf their resistance profiles.

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P30 | Clonal Outbreak of Multidrug-resistant Acinetobacter baumannii isolates harbouring the blaOXA-24/40 carbapenemase gene in an Intensive Care Unit in a Tertiary Hospital in Northern Spain

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Background

During the SARS CoV-2 pandemic a dramatic increase of patients requiring intensive care was triggered. The urgent and complex nature of these patients led to resource limitations, favouring the emergence of antibiotic resistant bacteria. In this study we investigated an outbreak of carbapenemresistant Acinetobacter baumannii (CRAb) in the ICU of our hospital.

Methods

From June to October of 2020, a total of 21 CRAb isolates involving 14 patients admitted to the ICU were collected (blood cultures (n=4); inguinal swabs (n=4); surgical wounds (n=2); urinary catheterization (n=2); pharyngeal swabs (n=1); and rectal swabs (n=1)), and 7 samples from the patient environment. Antimicrobial susceptibility testing was performed using the Negative Combo 38 Panel from MicroScan® WalkAway® 96 Plus System (both Beckman Coulter Inc.; California, USA). The isolates were sequenced using the MiSeq (Illumina) and the molecular epidemiology was studied using core genome MLST (cgMLST) based on 2390 genes (Ridom, Germany). Acquired resistance determinants were identified using ResFinder. Plasmid profiles were obtained by GeneJET Plasmid Miniprep Kit (Thermo Fischer Scientific) and by PFGE with S1 nuclease.

Results

Antimicrobial susceptibility testing showed values above the EUSCAST resistance breakpoints against all β-lactams (including imipenem and meropenem, MICs of ≥8 µg/ml) and susceptible for colistin (MIC ≤0.5µg/mL).

The blaOXA-24/40 gene was detected in all 21 CRAb. All isolates belonged to the same sequence type (ST) 2 (Pasteur)/ST801 (Oxford), harboured blaOXA-66 and were assigned international clone (IC) 2. Six A. baumannii were indistinguishable by cgMLST while the remaining isolates differed up to five alleles, thus they are considered as genetically related and part of the same outbreak. Four of the seven environmental isolates were identical to the major cluster (Figure 1). All isolates contained plasmids of 12,2 and 2,2 Kb, and 7 isolates an extra 32 Kb structure.

Conclusions

These data show that the emergence of blaOXA-24/40 is of high concern as no positive isolates had been detected in our hospital during the previous 10 years. Environmental samples containing blaOXA-24/40 highlight the need to control the reservoir of resistant isolates to prevent outbreaks.

P31 | Characterization of the subclass B3 Metallo-ß-lactamase ANB-1 from Acinetobacter nosocomialis

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Background. A carbapenem-resistant Acinetobacter nosocomialis was recently recovered from a rectal swab of a hospitalized patient. Whole genome sequencing (WGS) revealed the presence of a gene coding for NDM-1 along with a putative Metallo-ß-lactamase (MBL) gene, located on the chromosome of that strain.
The aim of this study was to further characterize this novel MBL and to determine its spread in that species.
Method. The blaANB-1 and its genetic environment were characterized by WGS (Illumina platform; Oxford Nanopore). Susceptibility testing was performed by broth microdilution and disk diffusion, according to EUCAST guidelines. A PCR-based screening of the carbapenem-resistant Acinetobacter spp. collection of our laboratory was performed. Phenotypic characterization was performed by heterologous expression in Escherichia coli TOP10 using pTOPO cloning vector.
Results. WGS analysis identified the A. nosocomialis isolate as belonging to ST224. The blaANB-1 encoded a 273 amino-acid long protein, sharing 42.5% amino-acid identity with the subclass B3 MBL CAU-1 isolated from Caulobacter crescentus, its closest relative. Retrospective analysis identified a blaANB-1-PCR positive signal in six out of the 233 (2.6%) screened Acinetobacter spp. in our collection. Sequencing of the PCR products identified two variants of the blaANB-1 gene respectively coding for ANB-2, and ANB-3 that share 99.3% and 93% amino-acid identity with ANB -1.
Expression of the three blaANB gene variants in E. coli TOP10 resulted in decreased susceptibility to several ß-lactams including ampicillin, piperacillin, ticarcillin, ceftazidime, cephalotin, cefotaxime and cefepime. In contrast, no reduced susceptibility was found for cefoxitin and carbapenems. No reduced susceptibility was observed for aztreonam and reduced susceptibility to the aformentioned ß-lactams was strongly inhibited by EDTA, in accordance with the known MBL properties. The ANB -2 variant exhibited the strongest activity. No mobile genetic element could be identified in the close vicinity of the blaANB genes.
Conclusion. ANB-1-like ß-lactamases are new subclass B3 MBLs with moderate activity against most ß-lactams and virtually no activity against carbapenems. Work is in progress to precisely evaluate their kinetic properties, the prevalence of blaANB genes in A. nosocomialis and other Acinetobacter spp species and the role these MBL genes might play in acquired resistance to carbapenems in that species.

P32 | Characterisation of Chloramphenicol Resistance Genes in Acinetobacter baumannii

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Acinetobacter baumannii is a multi-drug resistant Gram-negative bacterium of global concern. It commonly causes nosocomial infections such as ventilator-assisted pneumonia, bacteraemia, urinary tract infections, etc. It was recognized as the number one priority pathogen by the World Health Organization (WHO) in 2017 due to its multi-drug resistant status. A. baumannii is naturally resistant to chloramphenicol through a variety of different mechanisms such as regulation of membrane permeability, O-acetyltransferases and efflux pumps. Efflux pumps play an important role in antibiotic resistance and can convey drug resistance to multiple antibiotics or selective resistance to a single antibiotic. Two efflux pump genes craA and perM were previously investigated in A. baumannii and were shown to play a significant role in chloramphenicol resistance. However, the function, importance and regulation of these efflux pumps are poorly understood and there is conflicting evidence on which genes in A. baumannii provide the largest contribution to chloramphenicol resistance in A. baumannii. Transient chloramphenicol resistance was first identified in strain ATCC17978. In this study, the transient resistance phenotype was shown to be conserved in strain AB5075. Deletion mutants of craA and perM were constructed and characterised for their antibiotic resistance contribution in AB5075. We show that both genes play a role in intrinsic chloramphenicol resistance through antimicrobial susceptibility testing, but none of them appears to be the sole efflux pump responsible for chloramphenicol resistance, while CraA appears to play a crucial role in transient chloramphenicol resistance phenotype. PerM also appears to be involved in biofilm formation in nutrient deprived environmental conditions, suggesting that perM has a broader role for A. baumannii physiology than just drug efflux. An increase in motility was recorded upon the interruption of the craA gene. This preliminary data indicates that craA may be responsible for repressing motility. This study provides further insight into which genes are involved in chloramphenicol resistance in A. baumannii as well as a potential role for craA and motility and perM in biofilm formation.

P33 | Evaluation of novel immunological rapid test (Resist Acineto) Rapid Detection of acquired Carbapenemase Producers in Acinetobacter sp

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Background: Resistance to carbapenems in Acinetobacter spp. is a serious problem that is increasing worldwide. Almost all carbapenem-resistant A. baumannii strains produce an acquired class D (OXA-23, OXA-40, OXA-58) or class B (NDM, VIM, IMP) carbapenemase. The aim of this study was to evaluate the performance of the novel immunological rapid test, namely the “RESIST Acineto” (CORIS Bioconcept) for detection of the major acquired carbapenemases (OXA-23, OXA-40, OXA-58 and NDM) identified in Acinetobacter sp. Methods: A representative collection of 175 well-characterized carbapenemase and noncarbapenemase producing isolates belomnging to various Acinetobacter species from the Swiss National Reference Center for Emerging Antibiotic Resistance was tested, including 149 A. baumannii, 12 A.pittii, 4 A. radioresistens, 3 A. ursingii, 2 A. calcoaceticus, 2 A. nosocomialis, 1 A. bereziniae, 1 A. lwoffi and 1 A.junii. The acquired carbapenemase types were distributed as follows; OXA-58 (n=25), OXA-40 (n=31), OXA-72 (n=3), OXA-23 (n=37), and NDM (n=5). Noteworthy, 28 isolates co-produced two acquired carbapenemases, namely NDM + OXA-23 (n=10), OXA-23 + OXA-40 (n=6), NDM + OXA-40 (n=8), and NDM + OXA-58 (n=4). Results: Overall, the test performed very well with the exception of a systematic detection of an OXA-40/OXA-58 like carbapenemase for all the A. pittii strains tested, and an OXA-23 -like enzyme in Acinetobacter radioresistens. Nevertheless, it is actually known that A. pitii possesses a naturallyoccurring OXA-encoding gene type enzyme with the closest identity being OXA-40 while A. radioresistens expresses and OXA-23- type carbapenemase.

Therefore, after excluding results obtained for A. pittii and A. radioresistens, that can be considered as false-positive with respect to the identification of carbapenemase acquisition but must be considered as true positive properly speaking, this test showed an excellent specificity and sensitivity (100% and 100%, respectively, and positive and negative predictive values of 100%. Conclusions: The Resist Acineto test is a rapid, easy to perform, and showed an overall good specificity for detecting different variants of the four most common carbapenemases identified in Acinetobacter sp. Given its user friendliness, simplicity, and short time-to-result, this test is suitable for microbiology laboratories.

P34 | Dissemination of pan-aminoglycoside resistance gene armA and carbapenemase gene blaOXA-23 Acinetobacter baumannii Global Clone 2 in Switzerland, 2020-2021

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Objectives: Following the observation of an increased number of isolation of OXA-23 (carbapenemase) and ArmA-producing (16S RNA methylase conferring aminoglycosides resistance) A. baumannii at the national level in Switzerland, our aim was to evaluate whether some specific clone(s) may be spreading and/or emerging in Switzerland. Therefore, our study investigated and characterised all A. baumannii isolates harbouring both the blaOXA-23 and armA genes that were collected at the Swiss National Reference Center for Emerging Antibiotic Resistance (NARA) from 2020 to 2021. Methods: PFGE of 56 isolates submitted to NARA between 2020 and 2021 was realized and MLST was performed on at least one the representative of each PFGE profile. Whole genome sequencing (WGS) was performed on representative PFGE profiles using both the Illumina and Oxford Nanopore Technologies platforms. Susceptibility testing was performed on all 56 isolates by broth microdilution according to EUCAST guidelines.

Results: Between 2017 and 2021, 188 carbapenem-resistant A. baumannii were submitted to NARA and 70% (131/188) produced OXA-23 carbapenemase. Within these, the majority (n=98, 75%) also harboured armA. Fifty-six isolates from 2020-2021 were further analyzed.Nine PFGE profiles were identified and MLST on 11 representative isolates identified 3 different sequence types (one ST25, one ST1902, 9 ST2, that latter belonging to the Global Clone 2 (GC-2)). The blaOXA-23 genes were all found embedded within Tn2006 structures, as commonly described with GC-2 (ST2) isolates. Analysis of the six isolates subjected to long-read WGS identified that blaOXA -23 and armA genes were located on the chromosome in six and four isolates, respectively. Interestingly, two copies of the blaOXA-23 gene were present in 5 out of those 6 isolates. Other than aminoglycoside and carbapenem resistance, susceptibility testing revealed susceptibility levels to colistin at 94.6%, sulbactam at 1.8%, sulbactam-durlobactam at 87.5%, and cefiderocol at 83.9% or 91.1% (EUCAST or CLSI breakpoints).

Conclusions: Overall, this study identified that the A. baumannii co-producing OXA-23 and ArmA are increasing in incidence in Switzerland, largely due to the dissemination of the high-risk GC-2 clone. This highlights the importance of the continued surveillance of MDR A. baumannii strains, in order to reduce their potential further spread and ultimately, to preserve antimicrobial treatment options.

P35 | Insights into the genetic contexts of sulfonamide resistance among early clinical isolates of Acinetobacter baumannii

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Background
Since the late 1930s, resistance to sulfonamides has been accumulating across bacterial species including Acinetobacter baumannii, an opportunistic pathogen increasingly implicated the spread of antimicrobial resistance worldwide. Our study aimed to explore events involved in the acquisition of sulfonamide resistance genes, particularly sul2, among the earliest available isolates of A. baumannii.
Methods
The study utilized the genomic data of 19 strains of A. baumannii isolated before 1985. The whole genomes of 5 clinical isolates obtained from the Culture Collection University of Göteborg (CCUG), Sweden, were sequenced using the Illumina MiSeq system. Acquired resistance genes, insertion sequence elements and plasmids were detected using ResFinder, ISfinder and Plasmidseeker, respectively, while sequence types (STs) were assigned using the PubMLST Pasteur scheme. BLASTn was used to verify the occurrence of sul genes and to map their genetic surroundings.
Results
The sul1 and sul2 genes were detected in 4 and 9 isolates, respectively. Interestingly, sul2 appeared thirty years earlier than sul1. sul2 was first located in the genomic island GIsul2 located on a plasmid, hereafter called as NCTC7364p. With the emergence of international clone 1, the genetic context of sul2 evolved toward transposon Tn6172, which was also plasmid-mediated. Sulfonamide resistance in A. baumannii was efficiently acquired and transferred vertically, e.g., among the ST52 and ST1 isolates, as well as horizontally among non-related strains by means of a few efficient transposons and plasmids.
Conclusion
Timely acquisition of the sul genes has probably contributed to the survival skill of A. baumannii under the high antimicrobial stress of hospital settings.

P36 | Fast and efficient method for detection of Carbapenem Resistant Acinetobacter baumannii from screening samples

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Background:
Carbapenem resistant Acinetobacter baumannii (CRAB) is an important nosocomial pathogen. Early detection of CRAB carriers is required for infection control. Here, we evaluated several methods to detect CRAB from screening samples, including inoculation on selective chromogenic media and identification using blaOXA51 PCR.

Methods:
Our initial sample consisted of 125 randomly selected clinical isolates of A. baumannii. Meropenem MIC was determined by BMD and Agar Dilution. Identification was confirmed by gyrB and blaOXA51 PCR. Isolates were inoculated on three types of selective plates: SuperCARBA™, MDRAcinetobacter, and modified-MDR-Acinetobacter (4.5 mg/mL meropenem). The presence of blaOXA-23, blaOXA-24 and blaOXA-58 genes was determined by PCR.

Next, we prospectively examined the developed protocol by testing samples from 55 patients from a long-term care facility during a follow-up of CRAB outbreak; skin (after enrichment), rectal and sputum were sampled.
In addition, we carried out a series of experiments to determine the optimal enrichment time.
Results:
Of the 125 isolates, 70 (56%) were meropenem-susceptible, and 49 (39%) were CRAB. Sensitivity/specificity to detect CRAB were: SuperCarba 100%/0%, MDR-Acinetobacter 98%/78%, and the modified-MDR Acinetobacter, 92%/100%. The limit-of-detection of modified-MDR Acinetobacter was 102-103 CFU.
All A. baumannii isolates harbored gyrB and blaOXA51 genes, confirming it can be used for species identification. Of the CRAB isolates, over a half (29/50, 58%) carried an additional OXA carbapenemase: 13 harbored blaOXA23, 10 harbored blaOXA24, and 6/27 harbored both. None of the meropenem susceptible isolates carried these genes.

Among the 55 patients screened, 44 were identified CRAB carriers. Skin samples detected 97% (43/44) cases, rectal and oral samples detected 34% and 46%, respectively. Over-night skin sample enrichment in non-selective media improved detection (from 82% to 100%); 92% of positive samples were detected after 4 hours of enrichment.

Conclusions:
We present a procedure for fast detection of CRAB carriage using skin sampling, isolation on modified-MDR Acinetobacter selective medium after enrichment and, identification by gyrB and blaOXA51 PCR. Following this study, we used this protocol for large-scale screening and found it fast, reliable and easy to use.

P37 | A new MFS efflux pump and its putative regulator are involved in the antimicrobial resistance, virulence and surface-associated motility of Acinetobacter baumannii

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Background
Acinetobacter baumannii is a nosocomial pathogen classified by the World Health Organization as a priority bacterium for which new antimicrobials are urgently needed (1). Among the antimicrobial resistance mechanisms, efflux pumps are noteworthy, not only for conferring resistance but also for playing a role in the virulence of Acinetobacter spp. (2). In this study, we analyzed the oppositely contiguous genes A1S_3271 and A1S_3272 of A. baumannii, which are respectively annotated as a putative LysR transcriptional regulator and a putative MFS transporter.

Methods
Knockouts A1S_3271 and A1S_3272 of A. baumannii were generated by inserting a suicide plasmid into the target genes of the ATCC 17978 strain. To determine their distribution in A. baumannii strains, in silico analyses were carried out using the complete sequence of more than 500 genomes deposited in the NCBI database. Antimicrobial susceptibility was determined by both antibiogram and microdilution assays. Surface-associated motility was assessed on LB plates containing 0.5% agar, and virulence assays were conducted using the Galleria mellonella animal model.

Results
Our results point out that the LysR regulator is an activator of the MFS efflux pump, as knockouts of both the regulator and efflux pump exhibit increased susceptibility ranging from 2- to 4-fold to several antimicrobials, including rifampin, aminoglycosides, quinolones, tetracyclines, and trimethoprim. Interestingly, both genes are present in more than 90% of the analyzed strains, and their inactivation impairs surface-associated motility and decreases the virulence of A. baumannii.
Conclusions
The data reported here demonstrate that the newly characterized efflux pump, along with its putative activator, is widely distributed among clinical A. baumannii isolates and plays an important role in conferring antimicrobial resistance, motility, and virulence in this nosocomial pathogen. These findings underscore the potential of these proteins as putative new targets for developing novel therapeutic strategies against this pathogen.

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P38 | Surveillance, control and characterization of an NDM-1 Acinetobacter baumannii outbreak

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Background: NDM-producing carbapenem-resistant Acinetobacter baumannii (CRAb) are associated with nosocomial outbreaks, however, rarely in Germany. We have characterized a CRAb outbreak, investigated the source and routes of transmission, and successfully eliminated the pathogen from the hospital environment.
Materials/methods: Between May and September 2019, 11 CRAb isolates from ten patients were collected from four wards in the internal medicine department. Additionally, six CRAb isolates were obtained from extended environmental screening of high-touch surfaces and medical equipment. Antimicrobial susceptibility testing was performed using Vitek2/Etest (bioMérieux). Whole genome sequencing using the MiSeq (Illumina) and MinION (Nanopore) was performed. Genomes were analyzed using core-genome MLST (cgMLST) (Ridom) and in silico (Resfinder, Pasteur-MLST).
Results: 15 CRAb isolates encoding both blaNDM-1 and blaOXA-23 on the chromosome, and two with only blaOXA-23 were identified. One patient had two isolates; one co-harbouring blaOXA-23 and blaNDM-1, and the other blaOXA-23 only. All isolates harboured the intrinsic blaOXA-66 and were assigned international clone 2. All isolates were ST570. One isolate (blaOXA-23 only) was ST2 and considered unrelated. By cgMLST the blaOXA-23/blaNDM-1 isolates differed in ≤ 3 alleles, indicating inter-ward transmission. The ST570 blaOXA-23 isolate was identical to the blaOXA -23/blaNDM-1 isolates, and the difference in genotype was through loss of the Tn125-like encoding blaNDM-1. The index patient, transferred from a hospital in Egypt, tested positive for the outbreak strain on hospital admission, and although infection control and prevention measures were taken, patient-2, treated in another ward became infected four weeks later. Environmental sampling revealed diverse transmission reservoirs, i.e., electrical socket, electrocardiogram leads, and ventilator monitor. A two-stage cleaning of the affected wards, performed independently by two different cleaners, plus microbiological control afterwards, and improved hand-hygiene compliance of the staff resulted in complete eradication and ended the outbreak.
Conclusions: This study describes the transmission of a blaNDM-1-positive CRAb in the internal medicine department. The source of the outbreak was identified after environmental screening and was eradicated though strict infection control measures. These data highlight that the combination of high-resolution molecular surveillance (short- and long-read sequencing), extensive environmental screening, and thorough cleaning and disinfection, can effectively prevent further pathogen transmission in hospitals.

P39 | Development of an immunochromatographic lateral flow assay to rapidly detect OXA-23-, OXA-40-, OXA-58- and NDM-mediated carbapenem resistance determinants in Acinetobacter baumannii

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Background. Acinetobacter baumannii infections can be extremely challenging to treat owing to the worldwide prevalence of multi-drug resistant isolates, especially against carbapenems. 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. There is an unmet medical need to rapidly identify CRAb to enable appropriate antimicrobial treatment and to prevent transmission. Our aim was to expand the OXA-detection abilities of the rapid immunochromatographic test (ICT) OXA-23 K-SeT (Coris BioConcept, Belgium) to include OXA-40- and OXA-58-like carbapenemases, which together confer carbapenem resistance to more than 94 % of CRAb isolates worldwide.
Methods. We used hybridoma technology to generate monoclonal antibodies against OXA-40 and OXA-58 and selected them for productivity and specificity against recombinant and endogenous OXA-40 and OXA-58. Combinations of the resulting mAbs were analyzed in ICT format for their ability to detect recombinant rOXA-40His6 or rOXA-58His6, respectively. Subsequently, selected antibody-pairs were implemented into single-OXA-40 or single-OXA-58 prototypes and the final OXA-23/40/58/NDM ICT and were evaluated on clinical Acinetobacter spp. isolates with welldefined carbapenem resistance mechanisms.
Results. Five anti-OXA-40 and anti-OXA-58 mAbs, were selected. Competition ELISA with combinations of these antibodies revealed that the anti-OXA-40 antibodies bind to one of two binding clusters on OXA-40, while anti-OXA-58 antibodies bind to one out of four binding clusters on OXA-58. Direct binding to the corresponding antigen in an ICT format has left only three antibodies against rOXA-40His6 and rOXA-58His6, respectively for the subsequent sandwich ICT selection procedure, which revealed that the anti-OXA-40 (#5) and anti-OXA-58 (#A8) mAbs in combination with the cross-reactive mAb #C8 performed best. They were implemented into singleOXA-40 and single-OXA-58 ICT-prototypes and evaluated. These single ICT-prototypes demonstrated 100 % specificity and sensitivity.
Based on these results, an OXA-23/40/58/NDM-ICT was developed, complemented with OXA-23 and NDM-specific detection. An evaluation with selected carbapenem-resistant Acinetobacter spp. isolates (n=34) showed 100 % specificity.
Conclusion. With this easy-to-use detection assay, one can save 12-48 hours in diagnostics, which helps to treat patients earlier with appropriate antibiotics and allows immediate intervention to control transmission of CRAb.

P40 | Evolutionary drift of Carbapenem Resistant Acinetobacter baumannii in the Israel

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Background: Carbapenem resistant A. baumannii (CRAB) spread and dissemination poses critical clinical danger. CRAB sequence types (STs) 1 and 2 are most common, while ST3 is less widespread. In a recent study we identified that in Israel ST3 is a dominant clone, an unusual finding in other geographical locations. Here, we aimed to examine the prevalence of ST3 among CRAB clinical isolates over 23 years, and try exploring for clone characteristics over time.

Methods: We studied 148 random CRAB isolates from Tel Aviv Medical Center, isolated at four time points over the past 23 years (2000-2022). 41 were isolated during 2000-1, 48 during 2008-11, 42 during 2013-17 and 17 during 2022. The isolates were sequenced and typed by MLST. ST3 isolates sequencing were further analyzed to describe overtime changes in acquired genetic elements, and by FTIR for phenotypic clonality.
Results: The proportion of ST3 isolates among CRAB isolates increased overtime from 12.9% in 2000-1 to 47% in 2022. We identified a large, antibiotic-resistance IncP1α family plasmid carrying the qacE, tet(A), sul1, ant (2")-Ia genes among ST3 isolates. None of the 2000-2001 CRAB isolates contain the IncP1α plasmid. In contrast the majority of the ST3 strains isolated after 2013, carried this plasmid. In addition, the accessory genomes of recent ST3 strains contained on average a higher number of virulence factors, than the earlier isolates (108 in the 2000-1 isolates vs. 116 in the 2002 isolates). FTIR analysis showed multiple phenotypic patterns within ST3, with clustering of some isolates across different time points, suggesting that despite infection control efforts, successful clones persist over many years in the same hospital.
Conclusions: We found increasingly clonal dominance of ST3 CRAB isolates over 23 years period. This evolutionary success is associated with the acquisition of a a large, antibiotic-resistance IncP1α family plasmid, and with increase in the average number of accessory virulence factors. This dynamic reflects a gradual evolutionary change, likely in response to selective pressure giving rise to strains that are more adapted to survival in the clinical setting.

P41 | Acquisition of ciprofloxacin resistance is associated with increased biofilm formation and altered motility in acinetobacter baumannii

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Background: Acinetobacter baumannii is a non-motile opportunistic pathogen associated with mechanical ventilation pneumonia. Multidrug resistance (MDR) and biofilm formation emergence in A. baumannii represent a major clinical challenge. Biofilm formation can lead to motile biofilmdispersed microbes release, which may further infect other mucosal surfaces and/or enter the circulation. In this context, we investigated co-regulatory mechanisms of antibiotic resistance, biofilm formation, and increased motility as a means of increased bacterial virulence.

Methods: In this study was used 1) a ciprofloxacin susceptible A. baumannii SC1 (“domesticated” strain derived from ATCC 19606, 2) an isogenic-resistant mutant (CipR) of A. baumannii SC1 obtained by ciprofloxacin exposure (0,01 to 30 mg/L) in serial passages in LB broth during approximately 30 days and, 3) two MDR clinical isolates. The microdilution method was used to determine MIC. Biofilms were produced in the Calgary Biofilm Device (24 hours incubation). Biofilm biomass was evaluated by the Crystal Violet staining method and CFU/mL counting. Motility assays were performed in 0.3% agar media with 13 hours of incubation. XTT Cell viability assays were also performed. A. baumannii CipR and A. baumannii SC1 genomes were analyzed by Whole-Genome Sequencing (WGS).

Results: CipR (MIC of 512 mg/L) produced more biofilms than A. baumannii SC1. Clinical isolates demonstrated lower biofilm formation compared to CipR. CipR biofilm phenotype showed characteristic air-liquid interface and immersed mode of growth. CipR and MDR strains showed ditching/branching motility phenotypes. Whole-genome single-nucleotide-polymorphism analysis showed differences between CipR and A. baumannii SC1. Ciprofloxacin resistance is associated with altered biofilm phenotype and increased virulence.

Conclusions: Ciprofloxacin resistance promoted an increase in biofilm formation and concomitantly different motility phenotypes. This shows a novel relationship of antimicrobial resistance, motility ability, and biofilm formation. Further genomic studies will elucidate the mechanisms beyond resistance to ciprofloxacin, motility phenotypes, and increase in biofilm formation.

P42 | Polyclonal NDM producers Acinetobacter sp on Algerian hospital

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Métallo-béta-lactamases (MβLs) begin to play an important role in carbapenem resistance in Acinetobacter baumannii. A recent study, conducted in our hospital, estimates their prevalence at 13% of isolated CRABs (unpublished data). This work will aim to characterize at the molecular level, a collection of CRAB MβL(+) and to determine circulating clones in our hospital.

One hundred CRAB phenotypically characterized as MβLs (+) was collected over a period of 13 years (2006-2018) at the CHU Mustapha in Algiers. All of them benefited from a PCR search of NDM, VIM, IMP, GIM, SIM, SPM, DIM, FIM, AIM, KHM, and oxacillinases OXA-23, OXA-24, OXA -58 and OXA-51. Each strain, according to its molecular profile, was candidate for one or more of the following techniques: PFGE with ApaI (N = 40), OXA-51 base typing (N = 60), MLST according to the Pasteur scheme (N = 20). NDM-1 was the only MβL detected. Intrinsic OXA-51 was found in 98% of strains that are distinguished into four profiles:

Profile (P1) NDM alone found in 73% of CRAB MβLs (+): All showed an OXA-94 variant and an STP 85 clone. Six pulsotypes were distinguished by PFGE with a predominant profile (A) in 85% of the strains. Profile (P2) NDM + OXA-23 found in 13 strains: All showed an OXA-64 variant and an STP 25. Profile (P3) NDM + OXA-24 found in one strain that had an OXA-64 variant and an STP 25. Profile (P4) NDM + OXA-58 found in one strain that had an OXA-66 variant and an STP 63. Finally, a last profile (P5) was found in two strains NDM (+), but OXA-51 negative. This result indicates that these species are other than A.baumannii. The MLST analysis distinguishes them in two clones: STP1309 and STP1315. We report with this work, the first world description of STP 1315 clone. This study demonstrates the existence of five different clones of NDM producers Acinetobacter sp in our hospital. These clones being phylogenetically distant testify to probable genetic exchanges between these species.

P43 | Carbapenemase production in no fermentative Gram negative bacilli in Algeria

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Resistance to carbapenems in non-fermentative Gram negative bacilli (NF-GNB) involves very diverse mechanisms. If it is admitted that in A.baumannii it's the enzymatic mechanism which predominates. In the other NF-GNB, the production of carbapenemases is rarely found. This work aims to describe the type of carbapenemases detected in a collection of carbapem resistant NFGNB collected in an Algerian hospital.This is a prospective study conducted over 10 years (2012-2022) in Mustapha Bacha hospital of Algiers, where any carbapem resistant NF-GNB with a profile compatible with the production of a carbapenemase was included. For these strains, a β-carba test (Biorad®) was performed. Once positive, an EDTA phenotypic test was done to detect the possible production of a metallo-betalactamase (MβL). At the same time, research by PCR for the main carbapenemases was performed: KPC, NDM, VIM and IMP. The search for carbapenemases OXA-23, OXA-24 and OXA -58 was done systematically for A.baumannii.

A total of 400 carbapenem resistant A.baumannii (CRAB), 150 carbapenem resistant P.aeruginosa and 100 other carbapem resistant NF-GNB was collected. Among the 400 CRAB, all showed a positive β-carba test. PCR testing revealed that OXA-23 predominated at 77.5% (n=310), followed by NDM strains 9.5% (n=38), then OXA-72 at 9% (n=36). Strains co-producing two carbapenemases were found, in particular OXA-23+NDM (n=13), OXA-24+NDM (n=1) and OXA-23 +OXA-72 (n=2). Among the 150 carbapenem-resistant P.aeruginosa, only 31 (21%) showed a positive β-carba test thus producing carbapenemase. All showed a positive EDTA test. PCR testing revealed VIM-2 to predominate 77.5% (n=24), followed by VIM-4 19.4% (n=6). One strain co-produced VIM+IMP. Finally, among the 100 other carbapem resistant NF-GNB, only 4 (4%) showed a positive β-carba test, all confirmed as VIM-2 producers by PCR: Aeromonas hydrophila (n=2), Achromobacter xylosoxydans (n=1) and comamonas testosteroni (n=1).

These results demonstrate that the carbapenemases found in A.baumannii are largely distinguished from other carbapem resistant NF-GNB where only the metallo-beta-lactamase VIM is detected. Exception made for an IMP detection, described for the first time Algeria with this work.

P44 | Acintobacter baumannii: first etiology of nosocomial meningitis in the era of covid -19 at the university hospital of algiers

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Covid-19 has considerably changed the bacterial epidemiology of nosocomial infections. Nosocomial meningitis was no exception since we have seen an increase in cases over the past two years. The objective of this work is to report the etiologies of nosocomial meningitis described in the largest hospital in Algiers after the emergence of COVID-19. This is a retrospective study conducted over 2 years (June 2020 to July 2022) where all CSF referred to the clinical microbiology department were included. CSFs with positive cytology were distinguished according to their origin, the age of the patients and the bacterial etiologies. The bacteriological diagnosis being carried out exclusively by culture on enriched media. An Antimicrobial susceptibility testing was determined on MH according to CLSI criteria. During these two years, 1178 CSF were sent, which 86/1178 (7.30%) had positive cytology confirming the diagnosis of meningitis. This nosocomial meningitis having been found mainly in adults 47/86 (55%). These latter were hospitalized for the most part in neurosurgery at 53% (25/47) followed by ICU at 23% (11/47), forensic medicine at 11% (5/47). The remaining six cases occurred in medical departments such as gynecology, gastrology and pulmonology. Nosocomial meningitis in the pediatric population represented 45% (39/86). They were noted for the most part in neonatology at 79% (31/39) and in pediatrics for other cases 29% (8/39). Among the agents found, A.baumannii were the majority with 12 cases all occurring in neurosurgery. All the strains were multiresistant sensitive only to colistin. It is followed by 10 cases of Enterobacterales meningitis: K.pneumoniae ESBL and Serratia marcescens occurring in neonatology and ICU. In third position comes P.aeruginosa with 7 cases occurring mainly in neonatology (5 cases) and ICU (2 cases). It is followed by 4 cases of Enterococcus faecium occurring in paediatrics/neonatology, including one glycopeptide resistant (GRE). Others etiologies were isolated punctually: S.aureus, C.meningosepticum and yeasts. Finally, 23% (11/47) showed a negative culture corresponding to meningitis decapitated by prior antibiotic therapy. Multi-resistant NF-GNB represents more than half of the agents incriminated in our nosocomial meningitis. The broad-spectrum antibiotic therapies used since the emergence of Covid-19 have increased their involvement.

P45 | Acinetobacter baumannii: respiratory infections versus other clinical forms

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Acinetobacter baumannii is mainly responsible for respiratory infections in hospitals. However, in recent years we have witnessed an increase in invasive forms, particularly meningitis in neurosurgery and bacteremia in neonatology. The objective of this work is to determine the characteristics of respiratory isolates compared to those of other infections.

This is a prospective study conducted at the University Hospital of Algiers over 3 years (2015-2017) where any infection / colonization with A.baumannii has been clinically and microbiologically documented. All isolates benefited from a standard antibiogram following the recommendations of the CLSI, determination of the MIC of tigecycline by E-test® and of the MIC colistin by microdilution in liquid medium. A search by PCR for the main carbapenemases was carried out, in particular kpc, ndm, vim and imp. As well as OXA-23, OXA-24, OXA-58 and OXA-51 specific to A.baumannii. A total of 590 patients presented infection/colonization with A.baumannii including 175 respiratory forms (29.7%) and 154 invasive forms including 66 bacteremia (11.2%), 61 deep suppuration (10.3%), 12 meningitis (2%), 8 pleurisies and 7 ascitic fluid infections. The rest being represented by urinary forms (n=92), cutaneous colonizations/infections (n=121) and material colonizations (n=46).

Respiratory strains showed significantly greater resistance than other forms with 90% resistance to carbapenems, 95% to amikacin compared to 58% and 61% respectively for non-respiratory isolates. The MIC distributions of tigecycline and colistin were also significantly higher for the respiratory isolates. The molecular study reveals a clear predominance of carbapenemase OXA-23 for respiratory isolates (90%) strongly associated with armA methylase (in 68.3%). No-respiratory isolates show a greater diversification of carbapenemases with OXA-23 certainly predominant (70%) but also NDM (13%) and OXA-72 (12%) and a lower presence of armA methylase (31%).
An epidemic strain of A.baumannii OXA-23 + armA seems to be incriminated in the respiratory forms while the other forms would be due to isolates involving more diversified mechanisms.

P46 | Unraveling the Genetic Mechanisms of blaIMP-5 Dissemination in Acinetobacter Species: Insights and Unanswered Questions

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The IMP-5 metallo-b-lactamase was first described in Acinetobacter baumannii and later in Pseudomonas aeruginosa and Acinetobacter bereziniae. In these species, the blaIMP-5 gene was found embedded within a class I integron, flanked by two (MITE)-like elements. However, the genetic mechanisms contributing to the acquisition of blaIMP-5 remain poorly understood. In this study we aimed to explore the genetic platforms involved in the dissemination of blaIMP-5, in particular among Acinetobacter species. Four IMP-5-producing Acinetobacter isolates obtained from Portuguese Hospitals were included: A. baumannii Ac65 (urine, 1998), A. bereziniae HGSA93 (incubator water, 2008), A. bereziniae HGSA593 (blood culture, 2012), and Acinetobacter ursingii HGSA702 (drainage fluid, 2013). We assessed carbapenem MICs by E-test and conjugation assays (recipient strain: rifampicin-resistant A. baumannii ATCC17979) were conducted. A combined approach of Illumina and Nanopore sequencing generated high-quality hybrid assemblies using Unicycler. Gene cluster comparisons were visualized with Clinker and MOBsuite was used to predict mobilization modules.
All but one isolate displayed high carbapenem-MICs (>32mg/L). A. ursingii HGSA702 showed imipenem-MIC=1.5mg/L and meropenem-MIC=1mg/L. The blaIMP-5 was acquired by a defective Tn402-like class 1 integron, which was integrated per se (except for A. baumannii Ac65 that presented blaIMP-5 in the chromosome) into megaplasmids (>250 kb) displaying the MobP relaxase superfamily. Successful conjugation was observed for A. ursingii HGSA702, with transconjugants revealing an increase in imipenem (0.25mg/L to 8mg/L) and meropenem (0.5mg/L to 16mg/L) MICs, higher values that may reflect higher blaIMP-5 expression in A. baumannii species. All IMP-5-plasmids also encoded for additional b-lactamases such as OXA-58 and genes conferring resistance to aminoglycosides, macrolides, and heavy metals (e.g., mer, ars). A comparison of the Ac65 chromosome and the three blaIMP-5-megaplasmids revealed a common 25 kb- blaIMP-5 -associated region, as shown in Figure 1.
Here we provide important insights into the genetic mechanisms contributing to the dissemination of blaIMP-5 among Acinetobacter species, including the identification of a common 25 kb region that warrants further investigation. Despite the in silico prediction of conjugation machinery and the successful plasmid transfer, suggesting the potential for further plasmid dissemination, the limited occurrence of this carbapenemase, which seems to be confined to Portugal, remains a mystery.

P47 | Heteroresistance to cefiderocol in Acinetobacter baumannii is not a cause of the imbalance in mortality observed in CREDIBLE-CR

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Background
Heteroresistance is a phenomenon whereby communities of susceptible bacteria harbour a subpopulation of bacteria which may persist and grow under selective pressure but are unstable and revert when antibiotic is removed. Heteroresistance has been described previously in Acinetobacter baumannii (ACB) for colistin and it has been hypothesized that heteroresistance to cefiderocol may have contributed to the all-cause mortality (ACM) imbalance observed for ACB in the CREDIBLE-CR study. This study tested the ACB isolates from the CREDIBLE-CR study by population analysis profiling (PAP) to determine if any relationship between heteroresistance and mortality could be observed.

Methods
38 carbapenem-resistant ACB (CRAB) isolates from patients enrolled in the CREDIBLE-CR study were tested by PAP according to established methods with endpoints read after 72 hour incubation. The isolate was classified as resistant if the number of colonies that grew at the resistant breakpoint (16 mg/L) was ≥50% of those that grew on antibiotic free plates. The isolate was classified as heteroresistant if the number of colonies that grew at 32 to 64 mg/L were ≥1 in 106 of those on antibiotic free plates. If the isolate was neither resistant nor heteroresistant, it was classified as susceptible.

Results
In the CREDIBLE-CR study, 19 of the 38 patients with CRAB infections treated with cefiderocol died by day 49 (50% ACM). According to PAP (Figure 1), 47.4% (18/38) of the CRAB isolates were defined as heteroresistant, a similar frequency to that previously reported, however of those heteroresistant isolates the majority of patients 78% (14/18) survived, with only 4 isolates associated with deaths, fewer than those who died at Day 49 with susceptible (7) or resistant (8) isolates.

Conclusions
We found no correlation between the heteroresistant phenotype in CRAB isolates from cefiderocoltreated patients in the CREDIBLE-CR study and all-cause mortality at day 49. Frequency of heteroresistance was relatively high in CRAB but may be explained by biases inherent to the PAP method including non-iron depleted agar, high inoculum and prolonged incubation, all of which may decrease in vitro susceptibility to cefiderocol.

P48 | Emergence of multidrug-resistant Acinetobacter baumannii isolates carrying blaGES-35/blaOXA-23 and blaGES-11/blaOXA-23 genes in Alexandria, Egypt

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Background

Acinetobacter baumannii is a nosocomial human pathogen relevant in health-care facilities due to the emergence of multidrug-resistant isolates, mainly resistant to carbapenems. Of global concern is the spread in clinical setting of carbapenemase-producing isolates. In recent years GES-type enzymes have been increasingly reported in A. baumannii in different countries, some of them with carbapenemase activity, being the Mediterranean area a hot spot.

Methods

We studied 9 blaGES PCR positive isolates obtained in hospitals from Alexandria, Egypt. Antibiotic resistance profile was determined by Minimal Inhibitory Concentration using VITEK® technology. Then, Multi-Locus Sequence Typing was performed to determine the clonal relatedness and Whole Genome Sequencing (Illumina miSeq) to analyse the resistome and virulome. Plasmid profiles were analysed by GeneJET Plasmid Miniprep Kit and S1-Pulsed Field Electrophoresis, and afterwards for specific location of the blaGES gene, DNA hybridization with a blaGES specific DIG-labelled DNA probe was performed. Finally, we carried out plasmid PCR-based Replicon Typing to identify the corresponding replicase genes.

Results

Resistance profile of the isolates is shown in Table 1. Six isolates belonged to IC5 (Pasteur ST158) and three to IC9 (Pasteur ST85) clones, and harboured blaGES-35 and blaGES-11 variants, respectively. In addition, IC5 clones contained combinations of blaOXA-65, blaOXA-23 and blaGES -35 β-lactamase genes and IC9 clones contained blaOXA-94, blaOXA-23 and blaGES-11 βlactamase genes. All isolates harboured blaADC-like genes. Plasmid analysis showed that all isolates contained plasmids in the range from 2,2 Kb to 70 Kb, and blaGES hybridization showed that blaGES-35 and blaGES-11 genes were both located in a plasmid of approximately 70 Kb and in the chromosome. Finally, Replicon Typing revealed the presence of two types of replicases, Aci6 and p2S1.

Conclusions

Multidrug-resistant A. baumannii isolates were found in Alexandria hospitals harbouring different combinations of β-lactamase genes: blaOXA-65, blaOXA-94, blaOXA-23, blaGES-35, blaGES-11 and blaADC-like. All isolates carried different size plasmids. Especially remarkable was the presence of blaGES-35 and blaGES-11 genes in a plasmid of approximately 70 Kb and also in the chromosome.

P49 | Genomic analysis of tigecycline-resistant and extensively drug-resistant Acinetobacter baumannii harbouring a conjugative plasmid containing aminoglycoside resistance transposon TnaphA6

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Background: The occurrence of multidrug-resistant Acinetobacter baumannii (MDRA) has increased rapidly and is associated with severe nosocomial infections. MDRA has emerged in the hospital setting and has evolved into extensively drug-resistant A. baumannii (XDRA). Methods: A clinical XDRA isolate from a hospitalised patient in 2016 was evaluated for antibiotic susceptibility. Susceptibility was determined using the Clinical and Laboratory Standards Institute (CLSI) breakpoints. To detect acquired resistance, intrinsic resistance, and virulence genes, wholegenome sequencing was performed. Results: The carbapenem-resistant A. baumannii isolate was resistant to β-lactams, including broad-spectrum cephalosporins and carbapenems, and to aminoglycosides, fosfomycin, fluoroquinolones, tetracyclines, and trimethoprim/sulfamethoxazole, confirming the XDRA phenotype. The XDRA isolate harboured abaF, ant(3")-II-c, aph(3")-Ib, aph(6)-Id, armA, blaADC-73, blaTEM-1, blaOXA-66 and blaOXA-23, mphE, msrE, and tet(B). Quinolone resistance was associated with mutations gyrA S81L and parC S84L. Tigecycline resistance was also associated with a mutation in adeS, a sensor kinase of the two-component system AdeRS, which controls the AdeABC efflux system. The isolate belonged to Oxford and Pasteur scheme sequence type (ST) 1050 and 2, respectively, and harboured a conjugative plasmid containing the aminoglycoside resistance transposon TnaphA6. Conclusions: We found molecular resistance markers in an XDRA isolate and gave a genomic overview of resistance, virulence, and isolate origins. The isolate is closely related to a recent MDRA identified in Australia and the USA. This XDRA isolate is of concern in hospital and community care settings.

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Topics 5 – 7

P51 | Differential activity of Rho and CsrA in subpopulations of Acinetobacter baumannii regulate a switch between virulent and avirulent states.

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Background. Acinetobacter baumannii can rapidly interconvert between virulent (VIR-O) and avirulent (AV-T) subpopulations, distinguished by their colony opacity phenotypes. On laboratory media, the rate of VIR-O to AV-T switching in either direction is typically 10-20%. We have recently shown that this virulence switch is controlled by four TetR-type transcriptional regulators (TTTRs) that are stochastically activated in a subpopulation of VIR-O cells to drive them to the AV-T state. VIR-O cells can activate these TTTRs in alone or in different combinations to switch to the AV-T state, but the mechanisms controlling the activation of the TTTR's was previously unknown.

Results. We have demonstrated that differences in TTTR expression between VIR-O and AV-T cells is controlled by Rho-dependent transcriptional termination within a long mRNA leader region of each TTTR. In VIR-O cells, Rho-mediated transcriptional termination keeps TTTR expression in the OFF state. In a subpopulation of VIR-O cells, Rho-mediated termination is inhibited, allowing for TTTR expression and a switch to the AV-T state. Treatment of cells in the VIR-O state with the Rho inhibitor bicyclomycin resulted in activation of TTTR expression and a conversion to the AV-T state. However, a central unanswered question was how differences in Rho-mediated transcriptional termination at each TTTR was mediated. We now demonstrate that the ability of Rho to mediate termination at each TTTR is controlled by the RNA binding protein CsrA, which binds the TTTR mRNA leader regions and interferes with Rho-mediated termination. Loss of csrA prevented VIR-O to AV-T switching and overexpression of csrA greatly increased the rate of VIR-O to AV-T switching. We will present data supporting the hypothesis that the VIR-O to AV-T switch is directly controlled by the active/inactive state of CsrA. This activity mediated by differential activity of the two-component system GacSA in each cell subpopulation and the downstream expression of sRNAs that control CsrA activity.

Conclusions. The high-frequency switch between virulent and avirulent states is controlled by CsrAmediated interference with Rho-dependent transcriptional termination in the mRNA leader regions of four of TTTRs. The ability to regulate bacterial subpopulations by the combinatorial activation of a family of transcriptional regulators represents a novel mechanism in bacteria.

P52 | Csu pili dependent biofilm formation and virulence of Acinetobacter baumannii

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Acinetobacter baumannii has emerged as one of the most common extensive drug resistant nosocomial bacterial pathogens that causes a diverse range of human infections. Not only can the bacteria survive in hospital settings for long periods, but they can also resist adverse conditions such as exposure to antimicrobial drugs and disinfectants and long-term desiccation. However, underlying regulatory mechanisms that allow A. baumannii to cope with these conditions and mediating its virulence are poorly understood. Here, we demonstrate that bi-stable expression of the Csu pili, along with the production of poly N-acetyl glucosamine, regulates the formation of mountain-like biofilm-patches on glass surfaces to protect bacteria from the bactericidal effect of colistin. Csu pilus assembly is found to be an essential component of mature biofilms formed on glass surfaces and of pellicles. By using several microscopic techniques, we show that clinical isolates of A. baumannii carrying abundant Csu pili mediate adherence to epithelial cells. In addition, Csu pili suppress surface-associated motility but enhance colonization of bacteria into the lungs, spleen, and liver of a mouse model of systemic infection. The screening of c-di-GMP metabolizing protein mutants of A. baumannii 17978 for the capability to adhere to epithelial cells identified GGDEF/EAL protein AIS_2337, here denoted PdeB, as a major regulator of Csu pilimediated virulence and biofilm formation. Moreover, PdeB was found to be involved in type IV pili regulated robustness of surface-associated motility. Our findings suggest that the Csu pilus is not only a functional component of mature A. baumannii biofilms but also a major virulence factor promoting the initiation of disease progression by mediating bacterial adherence to epithelial cells.

P53 | Genomics of Acinetobacter baumannii iron uptake

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Background: Iron is essential for the growth of nearly all bacteria due to its redox activity and its role in many vital metabolic reactions. During infection, the nosocomial pathogen Acinetobacter baumannii responds to the low availability of iron imposed by the host by exploiting multiple ironacquisition strategies. To date, six different gene clusters for active iron uptake have been described in A. baumannii, encoding protein systems involved in: i) ferrous iron uptake (feo); ii) heme-uptake (hemT and hemO), and iii) synthesis and transport of the baumannoferrin (bfn), acinetobactin (bas/bau), and fimsbactin(s) (fbs) siderophores (Antunes et al., 2011; https://doi. org/10.1016/j.resmic.2010.10.010). Several clonal lineages of A. baumannii, including ST2, ST1, ST79, and ST25, are responsible for most hospital outbreaks on a global scale, with ST2 being the most dominant type by far (Hamidian & Nigro, 2019; https://doi.org/10.1099/mgen.0.000306). We investigated the occurrence of different combinations of iron uptake gene clusters in a vast collection of A. baumannii genomes to determine if there was a correlation between the emergence of certain epidemic clones and their ability to overcome the extreme iron limitation imposed by the host.

Methods: A compilation of more than 1,000 A. baumannii genome sequences in the PubMLST database was mined in search for the presence of known iron uptake gene clusters. Possible correlations between the presence of specific patterns of iron uptake gene clusters and the clonal lineages were determined. Results: The feo, hemT, bfn and bas/bau clusters were highly prevalent across the genomes' dataset (>98% of isolates), whereas the hemO heme uptake system was detected in only a portion of the dataset (69%), being more prevalent in the most successful A. baumannii lineages (Figure 1). The fbs gene cluster was extremely rare irrespective of the STs (1%). Conclusions: A. baumannii is endowed with a broad iron uptake capability since the majority of isolates belonging to the most common STs carry all iron uptake clusters except fbs. Given the close link between iron uptake capabilities and in vivo fitness, the presence of multiple iron uptake systems, including hemO, may have contributed to the success of some A. baumannii clones.

P54 | The capsule of Acinetobacter baumannii: roles and regulation

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Acinetobacter baumannii is a Gram-negative bacterium ranked as one of the most concerning bacterial pathogens by the CDC and the WHO. Despite its clinical relevance, only little is known about its virulence and resistance mechanisms. However, the production of envelope determinants, including the polysaccharide capsule, is known to be critical to protect it from the host immune system, desiccation, and antibiotic treatment. Genes required for capsule biosynthesis and export are clustered in the capsule locus (K-locus). Over 237 different K-loci sequences have been identified, witnessing a wide diversity in capsule composition and structure between isolates. In addition, the regulation of capsule production generates phenotypic heterogeneity in clonal populations. Our work focuses on building a better understanding of the polysaccharide capsule's involvement in the virulence and resistance of A. baumannii in various contexts. We recently identified a natural mutant of the AB5075 strain showing a stable translucent phenotype. Bioinformatic analysis of the K-locus revealed the presence of an ISAba13 insertion sequence, interrupting the itrA gene encoding for the initial glycosyltransferase, a key protein for O-linked protein glycosylation and capsule biosynthesis. By using a colloidal silica gradient to semi-quantify capsule production we showed that this mutant is not virulent in vivo and is non-capsulated. Moreover, we demonstrated that this insertional inactivation is fully responsible for these phenotypes and that the non-capsulated and avirulent itrA::ISAba13 mutant can revert to a capsulated and virulent state upon scarless excision of ISAba13 in presence of polymyxins. We are now investigating how the presence or absence of the capsule impacts the resistance and virulence of A. baumannii. Indeed, during antimicrobial treatment and infection, its production can increase and could act as a barrier in the interactions with biotic and abiotic surfaces. Using different capsule mutants and clinical isolates, we are assessing their adherence to polystyrene plates. Preliminary results show that the level of capsule production does impact adhesion to this abiotic surface. The next step is to translate this observation to other surfaces and epithelial cells. This project aims at building a better understanding of capsule regulation and pathogenesis of A. baumannii.

P55 | Cryo-electron Microscopy Structure of the Zifanocycline-Bound Ribosome from Acinetobacter baumannii Reveals a New Potential Binding Site of Ribosome

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Zifanocycline (KBP-7072) is a novel third-generation tetracycline-aminomethylcycline, exhibits broad-spectrum activity against Gram-positive and Gram-negative bacteria, especially multidrugresistant Acinetobacter baumannii. It solves common resistance caused by drug efflux and ribosomal protection in older-generation tetracyclines. Here, the structures of the zifanocyclinebound and tigecycline-bound ribosomes from A. baumannii ATCC 17978 were determined at 3.1 and 2.9 Å resolution respectively by cryo-electron microscopy. The structure analysis indicates that zifanocycline and tigecycline both bind to putative A-site and secondary 30S subunit core site as reported previously, while they also bind to a new binding site at the tail of 30S ribosomal subunit. Metal coordination of Mg2+ ions and hydrogen bonds stabilize their binding. We then compare the differences in binding modes between zifanocycline and tigecycline in all three target sites, and found that two antibiotics bind to the third site in similar ways but bind to the first two sites in different ways. These differences suggest that side chain modifications to the tetracycline core may be able to raise interaction strength with the ribosome and avoid ribosomal protection. In addition, we observe opposite orientations in some bound ligands. Our work highlights how the C9 substituents of tetracyclines influence their binding modes to ribosome and how an antibiotic can bind to the same bacterial targets though greatly different and flexible pathways. It may provide optimization direction of tetracycline antibiotics by considering the diverse ligand binding modes.

P56 | Efficacy of melittin combined with antibiotics against carbapenemase-producing Acinetobacter baumannii clinical strains

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Background

Infections caused by carbapenemases producing strains are a healthcare problem worldwide. This makes urgent searching for new approaches to fight these infections. Melittin are one of the most main components of bee venom. The aim of this study was to evaluate in vitro the activity and in vivo the efficacy of melittin in combination with antibiotics against carbapenemase-producing A. baumannii clinical strains.

Material and methods

In vitro: Six carbapenemase-producing clinical strains were used. The MICs of imipenem, meropenem, colistin, rifampicin and melittin were determined by microdilution method (EUCAST). The cytotoxicity 50 (CC50) of melittin was determined by AlamarBlue® kit. The synergistic activity of melittin in combination to antibiotics was determined by time-kill assays, at CC50 and MIC concentrations, respectively. In vivo: an A. baumannii producing OXA-23 clinical strain was evaluated in C57BL/6J female mice. Groups of 5 mice were intraperitoneally inoculated with the minimum lethal dose of the strain (8.70 log10 CFU/mL). Treatment was initiated 2h post-inoculation and lasted 24 hours for carbapenems combinations and 72h for the rest of combinations. Animals were randomly assigned to the groups: a) Controls (infected, untreated); b) melittin (M, 3µg/ml/12h/intraperitoneally); c) imipenem (IMP, 30mg/kg/4h/24h/intramuscularly); d) meropenem (MEM, 30mg/kg/4h/24h/intraperitoneally); e) colistimethate sodium (CMS, 20mg/kg/8h/72h/intraperitoneally); d) rifampicin (RIF, 25mg/kg/6h/72h/intraperitoneally); e) IMP+V; f) MEM+V; g) CMS+V and f) RIF+V. Immediately after death or sacrifice, bacterial concentration in spleen and blood and mortality was analyzed. (U Mann-Whitney test, chi-square, p256 mg/L. The bactericidal and synergistic activity of the combinations is showed in Table 1. The efficacy of melittin plus antibiotics is shown in Table 2.

Conclusions

1. All the combinations showed activity against the carbapenemase-producing A. baumannii clinical strains.

2. Meropenem plus melittin was the most active one, showing bactericidal and synergistic activity against the six strains.

3. Colistin and rifampin combinations improved the antibiotics monotherapies in reducing bacterial concentrations.

P57 | Efficacy of N-desmethyltamoxifen alone and in combination with colistimethate sodium and tigecycline in experimental pneumonia model caused by Acinetobacter baumannii clinical strains.

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Background:
Drug repositioning is an alternative in the development of new antimicrobials against infections by multidrug-resistant A. baumannii. We evaluate the efficacy of N-desmethyltamoxifen (N-DTAM) alone and in combination with antibiotics in an A. baumannii pneumonia murine model.

Methods:
Two clinical strains of A. baumannii: Ab#9 (tigecycline susceptible) and Ab#186 (tigecycline resistant). C57BL/6 mice females were inoculated intratracheally with minimal lethal doses of each strain (9 and 8 log10CFU/mL, respectively). Treatment began 2h after infection and lasted for 72h. Mice were randomly assigned to the following groups: a) controls (CTL); b) colistimethate sodium (CMS 20mg/kg/8h/intraperitoneally [ip]); c) tigecycline (TIG 5mg/kg/12h/subcutaneous); d) N-DTAM (40 mg/kg/24h/ip); e) N-DTAM+CMS; f) N-DTAM+TIG. Bacterial lungs concentrations, bacteremia and mortality were analyzed (U Mann-Whitney test, chi-square, $p<0.05$).

Results:
The efficacy of N-desmethyltamoxifen alone and in combination with colistimethate sodium and tigecycline is shown in the Table.

Conclusions:
1. Tigecycline in combination with N-desmethyltamoxifen was better than tigecycline alone in decreasing lungs and blood bacterial counts, bacteremia and mortality against both strains.
2. Colistimethate sodium in combination with N-desmethyltamoxifen was better than CMS alone in decreasing lungs and blood bacterial counts, bacteremia and mortality against A. baumannii #186 strain.
3. The results suggest that N-desmethyltamoxifen in combination with antimicrobials may be an therapeutic alternative against MDR A. baumannii.

P58 | Development of an Immunoinformatic Based Multi-Epitope Vaccine Against Acinetobacter baumannii

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Background:
Multi-drug resistant (MDR) Acinetobacter baumannii is an opportunistic pathogen associated with hospital acquired and combat related infections. Due to its environmental persistence, virulence, and limited treatment options, this organism causes increased patient mortality and healthcare costs. Prophylactic vaccination could be ideal for intervention against MDR Acinetobacter infection in susceptible populations. Immunoinformatic approaches, such as epitope prediction, have accelerated the design of potential vaccine candidates.

Methods:
In this study, we employed immunoinformatics to identify peptides containing both putative B and T cell epitopes from proteins associated with A. baumannii pathogenesis. A novel Acinetobacter MultiEpitope Vaccine (AMEV2) was constructed consisting of an A. baumannii thioredoxin A (TrxA) leading protein sequence followed by 5 identified peptide antigens. C57BL/6 mice were subcutaneously immunized with AMEV2 to evaluate its immunogenicity and protective efficacy. Antisera generated against AMEV2 was characterized via ELISA for antibody generation and in mechanistic studies with an opsonophagocytic killing assay. T cell and B cell reactivity to both the whole protein and UV-inactivated A. baumannii was determined with ELISpot. Protective efficacy was evaluated with an acute systematic infection model via intraperitoneal injection of the hypervirulent AB5075 strain.

Results:
Immunization with AMEV2 produced high antibody titers against the whole protein as well as individual peptide components. AMEV2 antisera were able to opsonize A. baumannii and enhance opsonophagocytic killing in vitro. Restimulation of vaccinated splenocytes with the AMEV2 protein resulted in both a Th1 and Th2 response with IFNγ and IL-4 secretion respectively, while exposure to UV-inactivated A. baumannii led to IL-4 secretion. Additionally, high frequencies of anti-AMEV2 secreting B cells were found in the spleen and bone marrow of vaccinated mice. AMEV2immunized mice were protected from a 4 LD50 systemic challenge at 5 weeks post final vaccination with a survival rate of 80%, while all mock vaccinated mice rapidly succumbed to infection within 2 days.

Conclusions:
The immunoinformatic designed multi-epitope vaccine, AMEV2, demonstrates robust immunogenicity and protection against A. baumannii. The ability of antisera to enhance the opsonophagocytic killing of the pathogen suggests antibody-mediated protection. Studies are currently underway to further dissect this antibody-mediated protective immunity.

P59 | Targeting iron homeostasis as a means to potentiate colistin treatment in MDR Acinetobacter baumannii

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Colistin, the last-line treatment for resistant A. baumannii, has been reported to be ineffective due to the emergence of resistance in clinical strains. Consequently, this has resulted in an exacerbated need for the discovery of compounds that can enhance the efficacy of colistin or overcome colistin resistance. In our study, we used high throughput screening to identify kaempferol, a natural plant phytochemical, as a potentiator of colistin activity in clinical strains of A. baumannii.

Colistin is known to induce ROS in bacteria through the hydroxyl radical pathway, which can contribute to bacterial cell death. However, A. baumannii can overcome exposure to the subinhibitory concentrations of colistin through the de-toxification activity of the iron dependant enzyme, superoxide dismutase B (SodB). By investigating the kaempferol mechanism of action, we found that it exposes bacteria to a metabolic vulnerability by disrupting iron homeostasis, through the inhibition of Fenton's reaction. This dysregulation of iron homeostasis results in a reduced Fe2+ availability, which is crucial for ROS detoxification by SodB. This eventually causes a build-up of toxic ROS within the cell, increasing susceptibility to colistin, resulting in cell death. Our results demonstrate that this vulnerability can be exploited to overcome both intrinsic and acquired colistin resistance in clinical isolates in vitro and in vivo in the Galleria mellonella model of infection. Overall, our study provides evidence that targeting iron homeostasis using natural products like kaempferol is a promising strategy for prolonging the lifespan of colistin and overcoming colistinresistant infections.

P60 | The artificial sweetener acesulfame-K inhibits growth of multidrug resistant Acinetobacter baumannii and potentiates carbapenem activity

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Antimicrobial resistance is one of the most pressing concerns of our time, as deaths caused by it and its burden to global healthcare systems are increasing at an alarmingly rate, resulting in the antibiotic resistance crisis. To tackle this, novel antimicrobial therapies are urgently needed. One possibility would be to identify antimicrobial activities in compounds whose safety for humans is well established, such as food additives. In this work, we screened a panel of artificial sweeteners searching for antimicrobial activity against multidrug resistant Acinetobacter baumannii AB5075. We selected acesulfame-K (ace-K) for further investigation, as it presented the strongest impact on bacterial growth. Next, we interrogated the antimicrobial and anti-virulence properties of ace-K on AB5075 at the gene expression level using dRNA-seq. One of the main changes in gene expression in the presence of ace-K was the downregulation of the pil and com genes, related to biogenesis and regulation of type IV pili. On the other hand, there was an upregulation of the paa phenylacetate degradation pathway, which was accompanied by the downregulation of the csu genes, responsible for biofilm formation. Subsequent phenotypic validation experiments confirmed ace-K can disable virulence factors related to these genes, such as, twitching motility, natural transformation and biofilm formation, respectively. Furthermore, sub-lethal concentrations of ace-K could potentiate the activity of carbapenems, drastically reducing their MIC for AB5075 (Figure 1A). Interestingly, genes encoding membrane-associated proteins were enriched in the dRNA-seq dataset, indicating ace-K can impact the cell envelope. To explore this impact as a potential mechanism of action, we performed live cell imaging experiments. The results confirmed this hypothesis for A. baumannii and an E. coli laboratory model, showing that the ace-K treatment leads to bulge-mediated cell lysis (Figure 1B). Finally, we challenged the therapeutic potential of ace-K in an ex vivo burn wound model using pig skin. As a result, an ace-K wash could decrease bacterial numbers in the AB5075 infected wounds more efficiently than a conventional chlorhexidine-based wash, as well as disassembling a preformed biofilm. Our findings provide consistent evidence supporting ace-K as a promising antimicrobial candidate or adjuvant against multidrug resistant A. baumannii.

P61 | Genome analysis of Acinetobacter strains with antifungal properties isolated from amphibians and from the nosocomial setting

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Background

The fungus Batrachochytrium dendrobatidis (Bd) is responsible for the extinction of many amphibian species around the world. However, it has been described that some bacteria that are part of the amphibian skin microbiome have antifungal properties and play a crucial role in the defense of these organisms against Bd. In this work we characterized, at the genomic level, eight Acinetobacter strains with antifungal properties from two species of Panamanian frogs Agalychnis callidryas and Craugastor fitzingeri.

Methods

Species identification was performed by paired ANI by contrasting the genomic sequence of our strains with those of the type strains. A phylogenetic tree was constructed with the nuclear genome. The ability to inhibit the growth of Bd and Botrytis cinera of all strains was evaluated. The antibiotic resistance profile of all strains and the physiological profile of strains belonging to novel Acinetobacter species were characterized.

Results

Genomic sequence analysis of the eight Acinetobacter strains isolated from frogs showed that five of them belong to already known species of the genus: A. pittii, A. radioresistens, and A. modestus. Surprisingly, we also identified three of them belonging to two undescribed species of the genus. We determined that seven of these strains inhibit the growth of Bd, but all of them are also able to inhibit the growth of the phytopathogenic fungus Botrytis cinerea, which is phylogenetically very distant from Bd. Genomic analysis of these strains suggests that the genes involved in the synthesis of antifungal compounds might be different in each of the Acinetobacter species analyzed. Since two of the strains studied with antifungal properties belong to A. pittii, we decided to evaluate the antifungal capacity of eight A. pittii nosocomial strains against Bd and Botrytis cinerea to determine if this property is exclusive of the strains isolated from frogs or if it is widespread. The results indicated that the nosocomial strains do not possess this property. We are currently analyzing the genomes of the strains that have this property and contrasting them with the sequence of those that did not have it, to identify candidate genes involved in the antifungal activity.

P62 | Antimicrobial, antibiofilm and antivirulence activity of glucocorticoid PYED-1 against Acinetobacter baumannii

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Background: Acinetobacter baumannii is a Gram-negative pathogen associated with multidrug (MDR) resistance and hospital outbreaks of infection. Due to increasing resistance to multiple classes of antibiotics and the ability of this bacterium to form highly structured and multilayered biofilms, A. baumannii infections are difficult to treat successfully. This makes the search for new antimicrobial strategies mandatory. Many natural steroids have been demonstrated to exhibit broad biological functions. Recently, the corticosteroid PYED-1 (pregnadiene-11-hydroxy-16α,17α-epoxy -3,20-dione-1) showed significant antimicrobial activity against bacterial and fungal pathogens. The aim of this study was to investigate the effects of a library of synthetic precursors of deflazacort (DFZ) on the growth of A. baumannii cells as well as on the formation and persistence of biofilms. Methods: The MIC values were measured by broth microdilution method, while the activity against bacterial biofilms was tested by crystal violet and tetrazolium salt reduction assay. The combination effects between PYED-1 and antibiotics were assessed by checkerboard assay. The transcription of selected virulence-related genes was verified by qRT-PCR. Results: The compounds were screened against A. baumannii reference strains and clinical isolates having different antimicrobial susceptibility profiles and belonging to different sequence types. Among all compounds, PYED-1 proved to be the most active molecule against A. bauamannii strains, showing rapid time-dependent kinetics of bacterial killing. Also, PYED-1 displayed an additive effect with colistin and amikacin against MDR A. baumannii isolates. Interestingly, the combination of amikacin and colistin with PYED-1 caused a reversal of antibiotic resistance. Then, we investigated the effect of PYED-1 on A. baumannii biofilms. Sub-MIC values of PYED-1 affected A. baumannii biofilm development in a dose-dependent manner, inducing a strong reduction in biofilm biomass and thickness. Also, PYED-1 disrupted preformed biofilms, decreasing both biofilm biomass and viability of A. baumannii biofilm cells. Transcriptional analysis was used to elucidate the mechanisms responsible for the inhibition of growth and biofilm formation. At sub-inhibitory concentration, the expression of biofilm- and virulence- associated genes was significantly suppressed after PYED-1 treatment. Conclusions: PYED-1 acts as a good antibacterial as well as antibiofilm agent and may represent a novel potential molecule for treatment of biofilm-related A. baumannii infections.

P63 | Bactericidal Efficacy Analysis of Silver Nanoparticles Synthesized from Bitter Gourd Extract

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The emergence of superbugs has made antibiotics increasingly ineffective, highlighting the need for alternative antibacterial methods. In this study, aqueous and ethanol extracts of No. 2 and No. 5 bitter melon, improved by Hualien District Agricultural Research and Extension Station of Taiwan, were used as reducing agents and modifiers to synthesize silver nanoparticles (AgNPs). The possible surface structure and antibacterial mechanism of AgNPs synthesized from bitter melon were investigated using ultraviolet-visible spectroscopy (UV-VIS) and Fourier transform infrared spectroscopy (FT-IR). Four clinical strains, Escherichia coli-K12, Staphylococcus aureus, Acinetobacter baumannii, and Pseudomonas aeruginosa, were used for antibacterial experiments. The AgNPs synthesized by aqueous bitter melon extract showed a stable and effective synthesis with an absorption peak in the visible light band of 400-420nm. FT-IR results indicated that the organic phase extract of bitter melon was a reducing modifier and was successfully adsorbed on AgNPs. All AgNPs modified with bitter melon exhibited good antibacterial effects, with No. 5 bitter melon exhibiting the best performance, including an antibacterial effect at higher dilution rates. The experiment was conducted using green chemistry methods, minimizing environmental damage by avoiding the use of harmful chemicals and maximizing the use of raw materials. This study contributes to the development of alternative antibacterial methods, emphasizing the importance of protecting the environment.

P64 | Bacteriocins as promising new weapon against the nosocomial pathogen Acinetobacter baumannii.

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Antibiotic resistance is at the origin of a 'silent pandemic' responsible of 1.27 million deaths in 2019, mainly due to six pathogens including the nosocomial pathogen Acinetobacter baumannii. Since there is an urgent need for new therapeutic strategies allowing for the eradication of antibiotic resistant and/or tolerant pathogenic strains, the efficiency of antimicrobial peptides including bacteriocins on A. baumannii must be explored. Bacteriocins are antimicrobial peptides or proteins (1 - 80 kDa) produced by bacteria. These small toxic proteins are secreted by the producer bacterial cell, which encodes an immunity protein, for interbacterial competition. Bacteriocins are very diverse in terms of sequence and structure, they can be post-translationally modified or not, exhibit narrow spectrum of activity (against member of the same species) or display broader activity spectra (against member of different species or genera). In this context, we tested 49 bacteriocins on A. baumannii clinical strains using different approaches including checkerboard assays, killing/time experiments and an adaption of Tolerence Detection test (TD test). We have shown that 11 bacteriocins are efficient against the very well characterized strain AB5075. Moreover, 9 of these bacteriocins have a synergistic activity with the last resort antibiotic colistin and we confirmed this phenomenon on recent clinical strains by TD test. Interestingly, we tested those combinations on a colistin resistant strain and showed that bacteriocins restore the antimicrobial activity of colistin. Our results show that bacteriocins have a real potential that needs to be further investigated, whether to treat contaminated surfaces (catheter, respiratory aid system) or infections caused by multiresistant strains of Acinetobacter baumannii.

P65 | Acinetobacter baumannii OmpA-like porins: functional characterization in bacterial physiology, antibiotic-resistance, and virulence

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Backgroud: Bacterial outer membrane proteins (OMPs) are directly involved in mitigating and adapting to changing environmental conditions to maintain cell homeostasis. In Acinetobacter baumannii, a prominent role in biofilm formation, eukaryotic cell infection, antibiotic resistance and immunomodulation is exerted by the outer membrane protein A (OmpA). Characterization of OMPs is essential to select potential candidates for new drugs and vaccine strategies for controlling this difficult-to-treat opportunistic pathogen. Therefore, this study aimed to elucidate the role of OmpAlike proteins of wild type (WT) A. baumannii AB5075 in fitness, antibiotic resistance, stress response and virulence. Methods: A collection of isogenic ompA-like mutants [ABUW_0505 (psaB), ABUW_1015 (carO), ABUW_2730 (arfA), and ABUW_3045 (yiaD)] were assayed for growth kinetics, antibiogram profiles, bacterial stiffness, surface hydrophobicity and motility assays. Permeability, cell envelope thickness, biofilm, cell adhesion, and in vivo virulence were also evaluated. The ompA mutant was included as the negative control. All results were analyzed in comparison to the WT. Results: Apart from ompA, no difference in the antibiogram profile were detected. All mutants displayed a growth defect; the rise in the temperature affected mostly psaB and arfA; intriguingly, psaB showed an increased cell stiffness and permeability. Indeed, psaB displayed a reduced cell envelope thickness with arfA and yiaD. Surprisingly, all mutants lost the twitching motility function. Together with ompA, arfA demonstrated a reduced biofilm-forming activity. All mutants but psaB were less adherent to lung epithelial cells; additionally, a decreasing gradient of virulence in the Galleria mellonella model was observed for all the mutants. Finally, the lack of PsaB, ArfA and YiaD significantly increased stress tolerance to human sera. However, the lack of PsaB results in increased tolerance to a number of different stresses. Conclusions: Taken together, these data indicate that PsaB has a crucial role in membrane fluidity that is critical in overcoming environmental stresses. ArfA contributes to the physiological cell envelope structure, aiding host cell adhesion. YiaD is involved in host-pathogen interactions. Hence, this study provides detailed clues about the role of not fully featured or unknown OMPs of A. baumannii.

P66 | Discovery of BfmR inhibitor in combination with Meropenem with Potent activity against Carbapenem resistance Acinetobacter baumannii

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The emergence and spread of Acinetobacter baumannii has posed a severe threat to public health, and its increasing resistance to existing antibiotics underscores the urgency for next generation of therapeutics. BfmR, the response regulator component of the two-component system, shows promising prospect as a novel antibacterial-target.

In this study, we aimed to discover BfmR inhibitors that may have a synergistic antibacterial effect when combined with meropenem. We performed a structure-based virtual screen for potential BfmR inhibitors. Three compounds designated 86、97、98 from this screen were identified, can restore multidrug-resistant strain ZJ06's meropenem sensitivity to MDRAB, and had moderate synergistic effects with meropenem. Compound 98 was able to downregulate the expression of K locus genes, which was similar to the BfmR knockout strain, indicating its role as a BfmR inhibitor. The scanning electron microscopy assay revealed that bacteria underwent morphological changes after treatment with these inhibitors. This study demonstrates a successful example of silico screening targeting BfmR to identify inhibitors and provides one promising lead compound for potential meropenem adjuvants for the treatment of MDRAB.

P67 | Acinetobacter baumannii is able to survive in natural soil for over four years

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Background:
The acute community-acquired human infections with Acinetobacter baumannii [1] suggest a source of this pathogen outside hospital settings. Survival of A. baumannii in natural soil depends on the soil pH [2] and in alkaline soil A. baumannii remained viable after five months of monitoring. However, the period when viable bacteria cannot be detected anymore in soil is not specified.

Methods:
Red palaeosol sampled in Istria, Croatia was poor in nutrients, of pH value of 8.43, free of carbapenem-resistant bacteria, but contained the native population of heterotrophic bacteria [2]. Three carbapenem-resistant isolates of A. baumannii, two environmental (EF7 and EF8) and one clinical (OB4138), were chosen for experiment. Isolates were separately suspended in the autoclaved commercial spring water. These suspensions were used to adjust the moisture of the fresh soil to maximum water holding capacity and simultaneously to supplement the soil with 6.7 ±0.3 log CFU/g of A. baumannii. Inoculated soil was left to dry in the dark at 22°C naturally, following drying in the desiccator upon the soil moisture dropped to 5 wt%. Concentration of viable A. baumannii was determined on selective CHROMagar Acinetobacter medium after incubation at 42°C/24h. Concentration of total heterotrophic bacteria was determined on nutrient agar after incubation at 22°C/72h.

Results:
Environmental isolate EF8 and clinical isolate OB4138 survived for two years and nine months, and were not detected after three years of monitoring (Fig.1). Environmental isolate EF7 survived for four years and one month, and was not detected after four years and five months of monitoring. Concentration of native heterotrophic bacteria decreased from initial 6.0 to final 4.9 log CFU/g.

Conclusions:
Once released in soil via human waste, isolates of A. baumannii remain viable for three to four years and represent the potential source of community-acquired infections. Responsible disposal of human waste is crucial to mitigate the dissemination of clinically relevant A. baumannii in soil.

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P68 | Evaluation of deaD as a component of the persistence molecular mechanism of Acinetobacter baumannii

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Acinetobacter baumannii is one of the main causes of healthcare-associated infections that threaten public health, causing great human suffering and a significant financial burden. Currently, A. baumannii infections are treated with carbapenems, such as meropenem; however, several cases of therapeutic failure have been reported in the last decade. That is mainly due to the presence of antimicrobial resistance by clinically relevant isolates of A. baumannii, as well as the presence of persister cells. Persisters constitute a fraction of the bacterial population that present a transient phenotype capable of tolerating supra-lethal concentrations of antibiotics. Nevertheless, the exact mechanism behind persistence has not been completely elucidated yet, but some proteins have been suggested as being involved in the onset and/or maintenance of this phenotype. DEAD-box RNA helicases are ATP-hydrolyzing enzymes that remodel RNA structure and RNA– protein complexes, but can also modulate environmental adaptation, host colonization and infectious processes. Thus, we investigated the expression of the deaD gene (RNA helicase) in A. baumannii cells before and after the exposure to meropenem. We found an increase of gene expression after 96 h (> 3-fold) and 144 h (>2.5 fold) of exposure to meropenem in planctonic cells. Similarly, we found an increase of expression of deaD (>3-fold) in biofilm of A. baumannii exposed to meropenem for 96 h. Since deaD contains an unusually long 5’ untranslated region that provides a tight control of gene expression through multiple regulatory mechanisms, it is possible that the gene products under its influence mediate adaptation to different environmental changes, such as tolerance to high doses of carbapenems by A. baumannii, in both planktonic and biofilm persisters. Finally, this data contributes to the understanding about the phenotypic features of A. baumannii persisters, as well as highlights DeaD as a potential target for drug development against A. baumannii.

P69 | Assembling the Acinetobacter baumannii surface: Exploring novel aspects of lipooligosaccharide synthesis

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A fundamental feature of the Gram-negative bacterial cell is the outer membrane (OM) that serves as a robust barrier against a variety of toxic compounds. The protection provided by the OM is predominantly due to asymmetry, with glycerophospholipids (GPLs) in the inner leaflet and lipopolysaccharide (LPS) or lipooligosaccharide (LOS) confined to the outer leaflet. LPS consists of a lipid A anchor, a core oligosaccharide, and a lengthy O-antigen. LOS, a truncated chemotype of LPS, lacks the O-antigen polymer. Although the biosynthesis of LPS/LOS is canonically essential for Gram-negative cell survival, Acinetobacter baumannii demonstrates a rare ability to survive in the absence of LOS. In conjunction with its non-fastidious growth and importance as a highly antibiotic-resistant nosocomial pathogen, A. baumannii serves as a uniquely relevant tool to study the OM. Experimental data examining the synthesis of the core oligosaccharide of A. baumannii LOS is limited despite its lack of archetypal phosphorylated heptose residues, which commonly provide a negative charge to fortify the OM. The effect this absence has on the cell's OM integrity is not currently understood. Even more interesting is an apparently novel mechanism of its third Kdo (3deoxy-D-manno-octulosonic acid) residue transfer. In all other organisms, the Kdo transferase KdtA (WaaA) is solely responsible for sequential addition of all Kdo residues in the core oligosaccharide; however, the discovery of a mutant possessing a Kdo2-chemotype, ΔlpsB, suggests that additional enzyme(s) are required for the transfer of the third Kdo residue. To uncover this unique mechanism of core synthesis, we characterized several A. baumannii 17978 core oligosaccharide mutants via tandem mass spectrometry and assigned putative functions to each gene in the predicted outer core locus. We identified a second mutant, ΔA1S_2903, that also shares the Kdo2 chemotype. Although loss of either the A1S_2903 or LpsB glycosyltransferases results in identical LOS chemotypes, the strains are phenotypically distinct. Still, both genes must be intact for complete core oligosaccharide synthesis. The data presented not only allows for better usage of A. baumannii as a tool to study OM integrity, but also provides further evidence for a novel mechanism of Kdo transfer and core oligosaccharide assembly.

P70 | Initial characterisation of the twin-arginine translocation system in Acinetobacter baumannii AB5075

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In bacteria, the twin-arginine translocation (Tat-) system exports folded proteins across the cytoplasmic membrane and the system has been shown to be important for several cellular processes including virulence. To date, the Tat system has not been characterised in the WHO priority pathogen A. baumannii. In A. baumannii, the Tat system is predicted to be composed of TatA, TatB and TatC. Computational predictions suggest that there are only very few Tat substrates, however, they include two phospholipases that are known virulence factors. Thus, we expect A. baumannii virulence to be affected in the Tat mutant. Expression of the tat genes in 12 different environmental conditions is very low, suggesting that the expression is tightly repressed or only required at a minimal level. Upon deletion of tatABC, A. baumannii shows a cell division defect which is strikingly similar to the phenotypes of other Tat mutants of Gram-negative bacteria such as Escherichia coli suggesting that the system is functional in A. baumannii. In E. coli, this phenotype is caused by the loss of periplasmatic amidases AmiA and AmiC, however, these enzymes do not appear to exist in A. baumannii. Thus, it is unclear how this phenotype is caused. We present an initial characterisation of the Tat pathway in A. baumannii, which shows conserved but also unexpected phenotypes and features compared to the best studied Tat system in E. coli.

P71 | An AT3 family acyltransferase participates in Acinetobacter baumannii nutrient metal acquisition and virulence

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Acinetobacter baumannii is a multidrug-resistant nosocomial bacterial pathogen that causes a range of diseases including respiratory and wound infections. A. baumannii is the leading cause of hospital-acquired pneumonia and has been identified as a major pathogen coinfecting COVID19 patients. The WHO has categorized A. baumannii as the most critical bacterial pathogen for the development of new therapeutics. Nutrient transition metals are essential to all life forms, including pathogenic bacteria. Vertebrates exploit this requirement by sequestering metals from invading pathogens in a process known as nutritional immunity. We have shown that the struggle for nutrient metals at the host-pathogen interface is a critical determinate of A. baumannii infection outcome. However, the mechanisms that A. baumannii employs to respond to, and overcome, nutritional immunity remain poorly understood. We have identified a gene, A1S_3410, which encodes for a membrane bound AT3 family acyltransferase that is induced during nutrient metal limitation. Our findings indicate that A1S_3410 targets cytoplasmic proteins for acyl-transfer, in contrast to AT3 acetyltransferase homologues which target extracellular carbohydrates. Loss of A1S_3410 results in a reduced capacity to survive in metal-limiting environments, diminished metal acquisition, and attenuated virulence in a mouse model of pneumonia. Collectively, these results suggest a previously unappreciated role of post-translational protein modification in the maintenance of bacterial metal homeostasis.

P72 | Transcriptional profiling of Acinetobacter baumannii during antibiotic and environmental stress

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Acinetobacter baumannii is recognized as a significant threat to human health as an opportunistic, multi-drug resistant, nosocomial pathogen. A secret to the performance of A. baumannii is the ability to quickly to adapt to changing environmental conditions by regulating gene expression programmes which includes genes that are essential. It is very important to understand the fundamental processes underlying antibiotic resistance and how A. baumannii responds to environmental stress in general. It now established that small regulatory RNAs and their RNAbinding proteins are key players in bacterial gene regulation. However, sRNAs have not been studied in detail in A. baumannii. Here we identify global expression patterns of A. baumannii AB5075 in 12 different conditions using RNA-seq. The growth conditions are: EEP (Early Exponential Phase), MEP (Mid Exponential Phase), LEP (Late Exponential Phase), ESP (Early Stationary Phase) and LSP (Early Stationary Phase), growth at different temperatures and different “shock” conditions: IRL (Low Iron), NaCl (Salt), CHL (Chloramphenicol), TET (Tetracycline) and COL (Cold Shock). Three biological replicates were sequenced from each condition, and after analysis of RNA-seq data and differential expression analysis throughout growth phases and environmental shocks, where MEP (Mid Exponential phase) was the comparator in all conditions, we identified the genes are up/downregulated. We observe large changes in gene expression when cells traverse from exponential into stationary phase and saw large transcriptomic changes when cells are exposed to the different environmental shocks, with the largest change in the antibiotic shocks. We found that the expression of sRNAs changes in response to several environmental shocks, and we confirmed the expression of sRNAs by Northern blotting. The results were largely consistent with those obtained from RNA-seq expression data. We focused on 6 small RNAs (sRNA44, sRNA99, sRNA100, sRNA75, sRNA76 and sRNA65) that exhibit interesting expression patterns (strongly down-regulated in different conditions). The study will deepen our understanding of transcriptional responses by A. baumannii in response to stressful conditions.

P73 | The protein HslJ boosts Acinetobacter baumannii survival against oxidative stress

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Backgroud: Acinetobacter baumannii is a life-threatening opportunistic pathogen that causes different infections, including ventilator-associated pneumonia. Due to its ability to sense and respond to the environmental stresses, A. baumannii can survive in hospital settings and persist during host infections. In a previous study, we identified the periplasmic protein encoded by the ABUW_2868 locus (hslJ gene) as being upregulated during imipenem exposure and hypothesized that it participates in the protective response against the oxidative stress. Methods: A hslJ mutant was attained from strain AB5075. Hydrogen peroxide assays and macrophage infections were used to determine the survival rates by colony forming units (CFU/ml) of the hslJ mutant in comparison with the wild type AB5075 strain. Surface hydrophobicity, biofilm-forming activity and motility in the hslJ mutant were also analyzed. hslJ transcriptional regulation is under evaluation using transactivation experiments and protein-DNA binding assay. Results: In comparison with the wild type strain, the absence of hslJ gene caused a significant drop in cell survival rates of the mutant strain both upon exposure to exogenous H2O2 and macrophage infection. Moreover, compared to the wild type strain, the hslJ mutant displayed a decrease in biofilm formation and motility abilities although had a higher surface hydrophobicity. Furthermore, preliminary results suggest that expression of hslJ is under the control of the master regulator in defense against oxidative stress, OxyR. Conclusions: The hslJ gene could be part of the regulon of OxyR. Being the HslJ protein located into the periplasm, it represents the first line of defense that confers resistance to oxidative stress that A. baumannii faces both in disinfectants used in hospital environments and innate immune cells during infection of the lungs. These findings provide a basis to develop novel therapeutics against this new target.

P74 | Differential expression of the AdeABC RND efflux pump and its regulator during motility in Acinetobacter baumannii

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Background: The RND efflux pump AdeABC which contributes to antimicrobial resistance in Acinetobacter baumannii is regulated by the two-component system AdeRS. Expression of adeRS is necessary for adeABC expression. We previously found using a LacZ reporter that AdeRS-ABC is constitutively expressed under different growth conditions except when motile. However, LacZ has a long half-life and is not suitable for single-cell studies. We have therefore investigated efflux pump expression at colony and single-cell level under different growth conditions using more suitable reporters. Methods: Expression of adeRS-ABC at colony level was investigated using a Lux and a sfGFP reporter systems. This was performed by putting the expression of the reporter gene under the control of the adeRS or adeABC promoter region, respectively, transformed into A. baumannii ATCC 17978, and assayed under planktonic, biofilm, and motility conditions.

1 To conduct single-cell confocal microscopy analysis, bacteria with adeRS tagged with sfGFP exhibiting the motile phenotype from the fringe of a motility plate, and non-motile phenotype from the center of the plate, were selected and fixed on microscope slides. DAPI was used to visualize bacteria on the slide. Results: The results of the luciferase biofilm and planktonic assays showed that adeRS and adeABC were constitutively expressed. On motility plates, bacteria at the site of inoculation (nonmotile) expressed both the regulator and the pump operons. Bacteria at the fringe (motile), did not express adeRS or adeABC. At the single-cell level, we observed that only bacteria picked from inoculation point were expressing adeRS (Fig1A). Slides prepared with cells from the migrating fringe did not express adeRS (Fig1B). Conclusion: At the single-cell level, these data have verified that adeRS is not expressed during motility, confirming previous observations using a less sensitive reporter at the colony level. These results highlight the importance of addressing the impact of growth conditions on efflux pump expression and suggest that motile bacteria are more susceptible to effluxed antimicrobials.

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P75 | Lights modulates resistance to desiccation in A. baumannii

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Acinetobacter baumannii has been recognized by the World Health Organization (WHO) as a critical pathogen. Research carried out by our group revealed that A. baumannii senses and responds to blue light modulating global physiological processes, both at moderate (23 °C) as well as at the normal temperature in mammals (37 °C). Moreover, it has been reported that A. baumannii can regulate multiple stress-related genes through the two-component BfmRS system.

In this work, we evaluated the effect of light on desiccation tolerance in different clinical A. baumannii strains: the multisensitive V15 and derived isogenic bfmRS mutants, as well as two clinical multirresistant strains recovered from patients. For this, bacteria were inoculated on filter papers and incubated under blue light or in the dark, at 37 or 23°C. Then, the number of surviving bacteria was determined. Our results indicate that A. baumannii exhibits photoregulation of desiccation tolerance. In fact, the number of V15 cells remained relatively constant until day 17 and reached undetectable levels by day 20 at 37°C. On the contrary, the number of viable bacteria decreased sharply from the beginning under light, falling below the limit of detection at day 4. Interestingly, ΔbfmR and ΔbfmRS mutants showed marked susceptibility to desiccation both under blue light and in the dark, behaving like the wild type under blue light. BfmS is not involved in the process, since ΔbfmS behaved similarly to the wild type. Complementation assays of ΔbfmR and ΔbfmRS with pBfmR rescued the responsiveness to light. Similar results were obtained at 23°C.

Clinical isolates showed higher persistence in the dark than under blue light, showing consistent phenotypes. In the dark, cell viability remained relatively constant for 28 days from the beginning of the experiment while under illumination conditions it fell below the limit of detection at approximately 10 days. In this study, we demonstrate that blue light, which is a safe, economical and accessible tool, inactivates different strains of A. baumannii under dessication conditions, behaving like a ΔbfmR mutant. Overall, blue light could be used to contribute to eradication of these microorganisms in hospital environment.

P76 | Contribution of Fourier Transform Mass Spectrometry to the study of the lipidome of Acinetobacter baumannii

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Regarding its interaction with environment, the membrane compartment of bacteria has a direct influence on the physiology. The modifications of lipid composition can have a direct impact on the membrane permeability and can influence the antibiotic penetration. In constant interaction with membrane proteins, they can also modify or inactivate their functions. Cardiolipins form distinct functional lipidic domains necessary to the activation of particular membrane proteins. Lipid direct modifications can lead to resistance to antibiotics. Due to the increasing number of therapeutic failures, Acinetobacter baumannii has become a global public health issue. In this context, lipid content can provide a better understanding of bacterial physiology and thus new therapeutic targets. The technology of choice for studying lipids is mass spectrometry. In this study, we tested recent ultra-high- resolution approaches to explore the A. baumannii lipidome. Thus, the lipidome of the reference strain ATCC 19606T was explored by two ultra high-resolution mass spectrometry approaches. The lipidome of lysed cells was first analysed by matrix assisted laser desorption ionization - Fourier transform ion cyclotron resonance (MALDI-FTICR) Bruker solarix 12 mass spectrometer. Via this approach, the resolving power (1,000,000, FWHM) allows the assignment of a unique molecular formula and lysed cells could be directly analyzed without laborious sample preparation steps. It allowed to detect, using 9-aminoacridine (9AA) around fifteen of the main phospholipids and, using 2,5-dihydroxybenzoic acid (DHB), the main cardiolipins and Lipid A. Moreover, after Bligh and Dyer lipid extraction, LC-ESI-MS/MS experiments with Orbitrap Exploris 120 allowed a semi-quantitative analysis. We highlighted the presence of about seventy phospholipids, i.e., of lysophosphatidyl-ethanolamines (LPE), phosphatidyl-ethanolamines (PE), phosphatidic acids (PA), phosphatidylglycerols (PG), Acyl-PGs, monolysocardiolipins (MLCL) and cardiolipins (CL). The acyl chain lengths can vary from 14 to 19 carbon atoms, the major being C16 and C18 ones exhibiting either none or one unsaturation, in agreement with previous study [1]. To conclude, these two methods are complementary: MALDI-FTICR allowed to have a global membrane lipidome overview by detecting together phospholipids and lipid A with few sample preparation and biological materials, when LC-MS/MS analysis allowed detailed information on phospholipid composition. Reference [1] Lopalco P et al., Nature.

P77 | Domain-architecture aware phylogenetic profiling indicates a functional diversification of type IVa pili in Acinetobacter baumannii

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Background

Comparative genomics approaches have successfully identified genetic changes that coincide with the emergence of pathogenicity in Acinetobacter. These methods typically rely on identifying either presence/absence patterns of genes, or changes in gene expression. However, screening for virulence-related factors does not routinely include the search for genes that might have undergone functional changes.

Type 4a pili (T4aP) are involved in a variety of functions, of which surface adhesion, bacterial motility, biofilm formation, and the uptake of environmental DNA are tightly connected to bacterial virulence. It is thus not surprising that several bacterial pathogens use T4aP for processes connected to the infection of the human host.

Methods

Using feature-aware phylogenetic profiling tool fDOG, we trace the protein set of A. baumannii ATCC 19606 across 855 Acinetobacter and 29 outgroup taxa. We investigate the prevalence of genes across pathogenic and a-pathogenic isolates, and the modification of evolutionary old and widespread proteins by comparing the feature architecture of orthologs, with a particular focus on T4aP components.

Results

We show that the pilus tip adhesin ComC exists with markedly differing domain architectures in pathogenic Acinetobacter species, annotating a von Willebrand Factor type A domain at the Nterminal region. The reconstruction of the ComC phylogeny suggests the emergence of three variants within A. baumannii, which along the pilins residing on the same operon, form an evolutionary cassette that has been replaced at least twice during A. baumannii diversification. The respective structures display variable arrangements of ~100 AA that form amidst the von Willebrand factor domain. Of particular interest is a finger-like protrusion that is characteristic of the main ComC variant, and we provide experimental evidence that this finger conveys virulence-related functions in A. baumannii.

Conclusion

The resulting strain-specific variants in the T4aP layout suggests differences in the way how individual strains interact with their host. Our study underpins the hypothesis that A. baumannii uses T4aP for host infection as it was shown previously for other pathogens. It also indicates that many more functional complexes may exist whose precise function can be rapidly adjusted by changing the domain architecture of individual proteins.

P78 | Alleles selected by growth in long-term stationary phase

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Acinetobacter baylyi can adapt to long-term stationary phase and display the Growth Advantage in Stationary Phase (GASP) phenotype. Genes induced during long-term stationary phase are required for a normal GASP response. Using whole-genome sequencing, we find alleles of hfq and putP selected for in aged populations. Small RNAs and transport of proline or sodium may be crucial for regulating gene expression during long-term stationary phase in Acinetobacter.

P79 | Repressor of the SOS Response Mechanism in Acinetobacter baumannii requires Helix-Formation and Dimerization for its DNA-binding Ability

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Following DNA damage, the multidrug-resistant pathogen Acinetobacter baumannii activates its SOS response, arresting the cell cycle to allow DNA repair and mutagenesis to occur by inducing error-prone polymerases. To repress its error-prone polymerase operons, A. baumannii employs the non-canonical repressor UmuDAb, a homolog of error-prone polymerase UmuD, along with its coregulator DdrR. Our overall goal is to determine how UmuDAb binds DNA to achieve this repression. A similar SOS repressor, LexA, requires dimerization to effectively bind DNA and repress transcription. Previously, we observed that disrupting either of two helices in UmuDAb's potential helix-turn-helix (HTH) domain impairs gene repression. Using a two-hybrid assay, and LexA-based predictions, we identified several amino acid residues and the C terminus as required for UmuDAb dimerization. To investigate if the UmuDAb HTH helices and its dimerization are required for DNA binding, we constructed and purified wild-type UmuDAb and UmuDAb proteins mutated in gene repression ability (HTH 1 and HTH2), amino acids required for dimerization in UmuD (N100D) or LexA (G124D), or the C-terminus (truncations W192X and R201X). We constructed fluorescent probes comprising a 37 bp UmuDAb binding site in the umuDAb-ddrR promoter region. Then, we performed electrophoretic mobility shift assays to observe how these mutations affect UmuDAb's DNA binding ability and analyzed their dissociation constants using non-linear regression with specific binding. UmuDAb bound to DNA tightly, with a Kd of ~35 nM. However, the various mutant UmuDAb proteins bound with greater than 8-fold lower affinity than wild-type UmuDAb, indicating that helices and dimerization are essential for UmuDAb to bind to DNA. We observed that mutants in the second helix of the HTH motif were more impaired in DNA binding than mutations in the first helix. The UmuDAb HTH2 mutant never achieved more than 54% binding to DNA. We also found that LexA-like dimerization site (G124D) was more important for binding than UmuD-like dimerization site (N100D) and that the C-terminus was crucial for DNA binding. Understanding how UmuDAb represses transcription of error-prone polymerases will further characterize DNA damage response mechanisms in A. baumannii and potentially uncover an exploitable feature for a novel therapeutic agent target.

Withdrawn Abstracts

ID Abstract: 4 | Poster: Characteristics of oral Acinetobacter spp. and evolution of plasmid-mediated carbapenem resistance in bacteremia patients with hematological malignancies

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Background: To characterize the genomic and evolutionary characteristics of oral Acinetobacter spp. in patients with hematological malignancies.

Methods: We analyzed the susceptibility phenotype and genomic characteristics of 48 oral Acinetobacter spp. and one bloodstream Acinetobacter baumannii from patients with hematological malignancies. PacBio sequencing and comparative analyses were performed to elucidate genomic evolution between oral colonization and bloodstream isolates from the same patient.

Results: A. baumannii was the most common Acinetobacter species in oral Acinetobacter spp. isolates, and the dominant global clones GC1 and GC2 were not present. 42 isolates were susceptible to carbapenems. One patient treated with meropenem for 15 days developed A. baumannii bacteremia 46 days after isolation of the oral A. baumannii AOR07. Oral and bloodstream isolates from the same patient were closely related with only four single nucleotide variations in the chromosome. The blaOXA-58 gene was shuttled from one plasmid to another by XerCD-mediated recombination and had an increased copy number, resulting in resistance to carbapenems in the bloodstream isolate.

Conclusions: The oral Acinetobacter spp. may be a source of bacteremia. Amplification and transfer of blaOXA-58 genes in plasmids explained the increased carbapenem resistance in bloodstream isolate.

ID Abstract: 7 | Poster: Genomic insights of Acinetobacter baumannii STPAS109/STOX1637 in Chile: Its path from carbapenem-susceptible to carbapenem-resistant

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Carbapenem-resistant Acinetobacter baumannii (CRAb) has been listed as 'top priority' by the World Health Organisation (WHO) for which new drugs are urgently needed. Although the global epidemiology of CRAb is dominated by the International Clones (IC) 1 to 9, in South America the prevalent lineages are STPAS15 (IC4) and STPAS79 (IC5) clones. Moreover, STPAS109/STOX1637, which is not part of the predominant ICs, has been detected in Chile. Due to the above, the aim of this study was to determine the genetic features of STPAS109/STOX1637 A. baumannii isolates collected between 1990 and 2012 in Chile.

Fourteen STPAS109/STOX1637 A. baumannii isolates recovered from human infections in Chile were analysed. Isolates were obtained in three different cities from the central-south region of Chile: Santiago (N=11), Concepción (N=2) and Valparaíso (N=1). Out of the 14, 10 isolates had been previously sequenced, whereas four of them were analysed for the first time in this study. Antibiotic resistance profiles were determined by the disk diffusion method. Species identification and blaOXA genes were analysed by multiplex PCR. Finally, we analysed the genomes of the 14 isolates by whole-genome sequencing or retrieving them from GenBank for those isolates sequenced previously.

All isolates were identified as A. baumannii and belonged to the STPAS109/STOX1637. Those isolates recovered during the 90s (N=3) were susceptible to carbapenems (CSAb), and resistant to cephalosporins, fluoroquinolones and aminoglycosides. Furthermore, those strains recovered from the 2000s (N=11) were resistant to all β-lactams tested (including carbapenems), sulphamethoxazole/trimethoprim, aminoglycosides, and fluoroquinolones. All isolates harboured the intrinsic blaOXA-67 (blaOXA-51-like allele) with no IS inserted upstream. The resistome of CSAb isolates was composed by genes mediating resistance to aminoglycosides, sulphamethoxazole/trimethoprim, chloramphenicol, and β-lactams; whereas the CRAb isolates possessed the same type of resistance genes and the carbapenemase-encoding gene blaOXA-58, which was flanked by ISAba3 (upstream) and IS26 (downstream) contained in a 17.4-kb plasmid belonging to the Rep3 Type-14. In conclusion, the evolution of STPAS109/STOX1637 from CSAb to CRAb in Chile is mediated by the acquisition of the blaOXA-58 gene contained in a Rep3 Type -14 plasmid favouring its maintenance and dissemination.

ID Abstract: 25 | Poster: Comparison of intestinal colonization of ST2 and ST23 Acinetobacter baumannii in mice

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Background: Acinetobacter baumannii is widely distributed in nature and hospital environment, and can be colonized in human skin, oral cavity, and gastrointestinal tract. Colonization of A. baumannii on human mucosal surfaces can lead to the formation of biofilms and increase the risk of bacteremia and respiratory infection.

Methods: Fifteen C57BL/6 mice were orally administrated with predominant clone ST2 or nonpredominant clone ST23 A. baumannii, respectively. Feces and gastrointestinal tissues of five mice were collected on every day after inoculation. The content of A. baumannii in the intestinal tract was quantitatively counted, and the differences in intestinal microbiome were analyzed.

Results: The overall positive rate of fecal culture in mice inoculated with ST23 was lower than that of mice inoculated with ST2. On the second day, all the five mice inoculated with ST2 were culture positive. However, only 2 of the 5 mice inoculated with ST23 were cultured positive, and the number of colonies was less than 50CFU/g. There were 26 OTUs specific to ST23, 40 OTUs specific to ST2 and 7 OTUs specific to the control group. The community composition analysis showed that compared with the control group, the proportions of Firmicutes and Bacteroidetes in ST23 and ST2 groups were decreased, and the proportions of Actinobacteriota and Desulfobacterota were increased. The proportion of Verrucomicrobia in ST23 group was significantly higher than that in ST2 group and control group. The results of intestinal culture showed that the positive rate of culture in large intestine (colon and cecum) was higher than that in small intestine (duodenum, jejunum and ileum). A. baumannii was detected only in colon and cecum on the first day of ST23 inoculation, while A. baumannii was detected in ileum, colon and cecum within three days of ST2 inoculation.

Conclusions: In mice with normal immune function, the intestinal colonization ability of ST23 was lower than that of A. baumannii predominant clone ST2 isolates. The colonizing sites were mainly located in the large intestine (colon and cecum). The increased proportion of Verrucomicrobia in intestinal flora of mice infected with non-predominant clone ST23 should be further studied.

ID Abstract: 79 | Poster: Crystal structure of the catalytic domain of the adenosine triphosphate phosphoribosyltrasferase enzyme (HisG) from Acinetobacter baumannii at 2.18A resolution

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Background: Histidine is an essential amino acid of living organisms, is not synthesized de novo in humans, and must be ingested. However, in the case of bacteria, histidine biosynthesis takes place de novo through a ten-step enzymatic pathway. Hence, the entire histidine biosynthesis pathway plays a vital role in the survival of microorganisms, and targeting the enzymes of this pathway can lead to the development of novel antibacterial therapeutics. ATPPRT catalyses the displacement on C-1 of PRPP by N-1 of ATP, leading to the formation of PR-ATP. ATPPRT is the first enzyme of this pathway and is actively involved in the regulatory role of the pathway. This makes ATPPRT a potential target for drug discovery. To develop novel antibacterial therapeutics against ATPPRT, its 3D structure needs to be elucidated. Methodology and Results: The structure of the catalytic domain of AbATPPRT enzyme at 2.18Å resolution was elucidated using X-ray crystallography. The crystals belong to orthorhombic space group P212121 with cell dimensions of a= 71.48Å, b= 79.20Å, c= 97.78Å, and α= =γ =90º. The structure was determined using the molecular replacement method and refined to several cycles of refinement and the final Rwork and Rfree factors were reported to be 19.8 and 25.3, respectively. The average B-factor was found to be 75.0 Å2. The structure shows the presence of two crystallographically independent molecules A and B in the asymmetric unit. The molecules A and B are arranged in the form of a homodimer which interacts in a head-to-tail fashion. Each chain displays two domains: domain 1 and domain 2. Domain 1 is formed by residues Met1-Lys111 and Ser196-Arg227 and has six β-strands and five α-helices arranged in the order β1-α1-β2-β3-α2-β4α3-β5-β11-α6-α7. Domain 2 includes residues Cys112-Val195 and has five β-strands and two αhelices. The two domains tend to form a crevice which is supposed to be the conserved active site area.

Conclusions: The 3D structure of AbATPPRT indicated the presence of a crevice which could potentially be targeted to inhibit the enzyme, thereby, inhibiting the pathway. Such compounds targeting the ATPPRT of the histidine biosynthesis pathway may become future potential drugs against Acinetobacter baumannii.

ID Abstract: 107 | Poster: The genetic context of resistance genes in extensively antibiotic resistant (XAR) Acinetobacter baumannii isolates at an NICU

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To characterize Acinetobacter baumannii isolates responsible for a 1 month outbreak at an NICU from a Tehran hospital, the seven isolates recovered from blood of neonates within the outbreak were included. The isolates were examined for antibiotic susceptibility, global clone (GC) by multiplex PCRs, antibiotic resistance genes (ARG), genomic resistance islands, and plasmids using PCR and PCR mapping. The Institut Pasteur Multi-locus Sequence Typing (MLST) scheme was used to determine the sequence type (ST).

The A. baumannii isolates were resistant to carbapenems, aminoglycosides except amikacin, thirdgeneration cephalosporins, and fluoroquinolones but they were susceptible to colistin. All but one isolates were resistant to sulfamethoxazole. The isolates belong to global clone 2 and ST2. All isolates carried the oxa23 within Tn2006, which Tn2006 was located in the AbaR4 and the AbaR4 was located within AbGRI1 resistance island in all the isolates. The blaTEM was carried by AbGRI2 -12b resistance island. The aphA1b gene was located within Tn6020, which it was carried by AbGRI2-12b. The armA and aadB genes were carried by AbGRI3-4 resistance island and pRAY*, respectively. None of the isolates contained aphA6. ISAba1 was found upstream of the ampC gene in all the isolates. These findings indicate that an outbreak caused by the XAR A. baumannii isolates belonging to the most globally distributed clone, GC2 that had acquired mobile genetic elements carrying ARG.

ID Abstract: 111 | Poster: Antibiotics and Acinetobacter

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A. lwoffii and A. baumannii are the leading causes of Acinetobacter-derived bacteraemia in England and Wales, 33% and 18% respectively (PHE, 2022). Whilst both species are similar in the infections that they cause, they have markedly different antibiotic susceptibility profiles (PHE, 2022). A. lwoffii is almost pan-drug sensitive, whereas A. baumannii is frequently multi-drug and even pandrug resistant, leading to life-threatening infections that are challenging to treat. It is currently unknown why these two species, that inhabit similar niches and cause similar infections, have such different responses to antibiotic treatment. Here, 4,809 A. baumannii and 38 A. lwoffii whole genome sequences were interrogated to investigate why A. lwoffii is more susceptible than A. baumannii. A. lwoffii encoded far fewer antibiotic resistance and efflux pump associated genes than A. baumannii explaining its greater antibiotic susceptibility. However, A. lwoffii encoded more DNA defense systems, indicating that A. lwoffii is more stringent about the DNA it maintains within its genome. Our results also show that A. lwoffii has a lower mutational frequency than A. baumannii and that A. lwoffii does not seem to acquire mobile drug resistance genes. Understanding if A. lwoffii might develop drug resistance and the mechanisms of this will enable the appropriate treatment of A. lwoffii infections and shed light on how multi-drug resistant pathogens, such as A. baumannii, may have developed resistance.

ID Abstract: 117 | Poster: Effect of intranasal Acinetobacter baumannii infection in the serum lipid profiles in mice

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Background: Acinetobacter baumannii is a multidrug-resistant bacterial pathogen and causes significant healthcare-associated infections with high mortality. Despite its medical importance, the pathogenesis and host interaction with this pathogen remains largely unknown, which impedes the successful development of novel vaccines and therapeutic interventions.

Methods: We analysed the serum lipid profiles in mice infected intranasally with A. baumannii using an LC-FAIMS-MS system. We first optimized the parameters using lipid standards. Results: The analysis of lipid extracts of the serum samples collected from controls and 24 hours post-infection was carried out using 3 separate CVs (e.g., 29 V, 34 V and 39 V) in a single LC-FAIMS-MS run. This CV-stepping experiment design can increase the selectivity in the detection of lipid classes. Our preliminary data indicated an increase in the overall abundance of phosphatidylethanolamine (PE) in infected mouse serum compared to control samples; whereas ether PE and lysophosphatidylethanolamine (LPE) abundance decreased following infection. Interestingly, both phosphatidylcholine (PC) and ether PC abundance increased in infected serum samples, but lysophosphatidylcholine (LPC) decreased due to infection. On the other hand, no significant changes in phosphatidylinositol (PI) abundance were detected. Conclusions: Intranasal A. baumannii infection has substantially changed serum lipidomic profiles, even at the early stage of the infection. The potential contribution of these changes to the pathogenesis of A. baumannii will be discussed in the context of their kinetics, bacterial burdens and other host factors.

ID Abstract: 166 | Poster: Role of Glu152Lys substitution in the enhanced cefiderocol hydrolysis of NDM-9 in A. baumannii

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Background: Cefiderocol is a new siderophore-conjugated cephalosporin with potent activity against multidrug-resistant (MDR) Gram negative pathogens including Acinetobacter baumannii. However, some beta-lactamases, such as NDM-type enzymes, have been shown to alter cefiderocol susceptibility of A. baumannii clinical strains. While some NDM-1-producing A. baumannii strains are susceptible to cefiderocol (MIC £ 2mg/L), the blaNDM-9 gene was identified in a A. baumannii clinical isolate highly resistant to cefiderocol (MIC > 32 mg/L). The aim of this study was to evaluate the role of NDM-1 and NDM-9 in cefiderocol resistance levels and to compare the steady-state kinetic parameters of the enzymes NDM-1 and NDM-9 with respect to this antibiotic.

Methods: The blaNDM-1 and blaNDM-9 genes were cloned into a pABEC vector and expressed in E. coli and A. baumannii reference strains. Susceptibility to cefiderocol was determined by the broth microdilution method. Steady-state kinetic parameters were measured using purified NDM-1 and NDM-9 enzymes. The role of the amino-acid change in the cefiderocol hydrolysis activity was explored by molecular docking experiments.
Results: NDM-9 enzyme production was associated with a higher MIC of cefiderocol compared to NDM-1 in both E. coli and A. baumannii. The affinity for cefiderocol was 3-fold higher for NDM-9 than for NDM-1. Molecular docking experiments indicate that residue 152 of NDM-type enzymes plays a key role in cefiderocol binding and resistance.

Conclusion: Our data confirm that the mutation Glu152Lys (present in NDM-9) results in increased cefiderocol-hydrolytic activity.

ID Abstract: 42 | Oral Communication: Genomic and phenotypic analyses of diverse non-clinical Acinetobacter baumannii strains reveals strain-specific Virulence and resistance capacity

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Acinetobacter baumannii is a critically important pathogen known for its widespread antibiotic resistance and ability to persist in hospital-associated environments. Whilst the majority of A. baumannii infections are hospital-acquired, infections from outside the hospital have been reported with high mortality. Despite this, little is known about the natural environmental reservoir(s) of A. baumannii and the virulence potential underlying non-clinical strains. Here, we report the complete genome sequences of six diverse strains isolated from environments such as river, soil, and industrial sites around the world. Phylogenetic analyses showed that four of these strains were unrelated to representative nosocomial strains and did not share a monophyletic origin, whereas two had sequence types belonging to the global clone lineages GC1 and GC2. Further, the majority of these strains harboured genes linked to virulence and stress protection in nosocomial strains. These genotypic properties correlated well with in vitro virulence phenotypic assays testing resistance to abiotic stresses, serum survival, and capsule formation. Virulence potential was confirmed in vivo, with most environmental strains able to effectively kill Galleria mellonella greater wax moth larvae. Using phenomic arrays and antibiotic resistance profiling, environmental and nosocomial strains were shown to have similar substrate utilisation patterns although environmental strains were distinctly more sensitive to antibiotics. Taken together, these features of environmental A. baumannii strains suggest the existence of a strain-specific distinct gene pool for niche-specific adaptation. Furthermore, environmental strains appear to be equally virulent as contemporary nosocomial strains but remain largely antibiotic sensitive.

ID Abstract: 93 | Oral Communication: Rational strategy of plant associated Acinetobacter for their mutualistic co-evolution

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Environmental Acinetobacter calcoaceticus strain P23, a symbiont of aquatic Lemnaceae plants, significantly enhances the growth of host plants by unique mechanisms. P23 adheres primarily to the frond, leaf like structure part, surfaces and forms flat and wide biofilms. Extracellular polymeric substances of P23, adhesive matrix of the biofilm, are composed of exopolysaccharides like many bacteria, EPS-P23. Interestingly, the addition of EPS-P23 to the plant culture medium significantly accelerated the growth of Lemna minor. When the sugar composition of EPS-P23 was analyzed, glucose and galactose were found in addition to the general bacterial exopolysaccharide component, N-acetylglucosamine. As a result of P23 draft genome analysis, at least three glycosyltransferase gene clusters were found. Among them, gene cluster P23_2757/2761, which is shared only by several Acinetobacter strains. P23_2759/2761 was heterologously expressed in Acinetobacter baylyi ADP1, which is unrelated to the plants. The recombinant A. baylyi ADP1 (pBBR1-P23_2759/2761) exerted apparent plant growth-promoting activity for L. minor. These results indicate that the mutualistic A. calcoaceticus P23 had co-evolved with the host plant and created its own exopolysaccharide EPS-P23. EPS-23 is capable of not only stabilizing the biofilm but also increasing the number of host plants so that P23 successfully may expand its comfortable habitat.

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Y. Yamakawa et al. (2018) Plant Growth Regul. 86(2), 287-296.
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ID Abstract: 94 | Oral Communication: The Journey from Pathogenesis to Products: Exploiting Acinetobacter baumannii Iron Acquisition with Monoclonal Antibodies to Prevent Infection

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Background

Antibiotic resistance is a global concern, and according to the WHO, there are only nine new antibiotics in the pipeline for Acinetobacter baumannii. Therefore, new therapeutics, should be developed to enhance the current armamentarium. Monoclonal antibodies (mAbs) provide a treatment modality that has been clinically successful but not fully exploited in the antibacterial space. We developed a gated-tiered approach to screen mAbs, down-selecting for therapeutic development. Using this approach for A. baumannii, two candidate mAbs (αBauA and αOmpW2) were identified, which targeted surface proteins required for iron acquisition in the host.

Methods

In vitro: αBauA and αOmpW2 were developed against recombinant GST-fusions. Native ELISA screened a genetically diverse set of 100 A. baumannii strains. Bacterial growth inhibition was measured via optical density in iron-deficient medium. An opsonophagocytic assay was developed with pHrodo-labelled A. baumannii; and mAb-mediated opsonophagocytosis was measured by fluorescent intensity.
In vivo: Galleria mellonella, mouse pulmonary and mouse wound models were used to evaluate the efficacy of αBauA and αOmpW2. G. mellonella larvae or mice were injected systemically with each mAb, the combination, or isotype control. Animals were challenged with AB5075. In the murine models, mice were infected intranasally or directly into the wound bed. After infection, larvae and mice were monitored for survival and other secondary endpoints.

Results

αBauA and αOmpW2 recognized the native surface of A. baumannii (>68% isolates). These mAbs also reduced bacterial growth alone and in combination. αBauA and αOmpW2 did not have opsonophagocytic activity. In vivo results demonstrated that in combination, mAbs promoted 70-100% survival in all models. In the wound model, faster healing and less inflammation was observed.

Conclusions

The combination of αBauA and αOmpW2 synergistically inhibits A. baumannii growth in low iron medium and disrupts pathogenesis in vivo. This suggests that BauA and OmpW2 are required for iron acquisition, but because no opsonophagocytic activity was observed, the mechanism of action appears to be solely anti-virulence. Prophylactic protection in three separate animal models suggests multiple indications could be pursued. Future work includes humanization and dosing optimization in combination with antibiotics.

ID Abstract: 132 | Poster: Structures and dynamics of Intensive Care Unit Acinetobacter baumannii populations

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Background
Carbapenem-resistant Acinetobacter baumannii (CRAB) is a significant public health burden. To develop effective infection prevention and control (IPC) strategies, it is crucial to understand hospital A. baumannii communities. We characterised the A. baumannii populations of a 28-bed intensive care unit (ICU) in Hangzhou, China, over two three-month periods in 2019 and 2021.

Methods
Sampling was conducted weekly for 13 weeks in each study period. Oral, rectal, and indwelling tube swabs were collected from all ICU patients. All bed unit, sink, and equipment surfaces were swabbed. All A. baumannii from clinical specimens were obtained. Between study periods, IPC practices were introduced in response to the 2019 study and COVID-19.

MICs for imipenem, meropenem, amikacin, tigecycline and colistin were determined. 1,072 isolates (971 CRAB) were whole-genome sequenced (Illumina), and 120 were long-read sequenced (Oxford Nanopore). The population was partitioned into clusters following phylogenetic analyses. Plasmid, transposon and resistance island structures were determined from complete genomes.

Results
In August-October 2019, 547 of 551 (99.3%) CRAB isolates were OXA-23 carbapenemaseproducing global clone 2 (GC2). However, the GC2 population was diverse, comprised of 17 phylogenetic clusters that had been introduced to the ICU independently. We observed clusters being introduced by new patients, appearing in multiple ICU rooms, and being acquired by previously CRAB-negative patients. Patient acquisition was strongly associated with environmental contamination. Distinct CRAB clusters were often co-located, and we observed three plasmid transfer events.
In May-July 2021, GC2 accounted for just 50.8% of CRAB (213/419), and these isolates were largely distinct from the 2019 clusters. Fourteen GC2 clusters were cleared from the ICU within three weeks of introduction, while one was present throughout the study, accounting for 10/14 clinical isolates. The remaining 206 CRAB were a novel ST164 strain that produced OXA-23 and NDM-1. ST164 persisted throughout the study and appeared to have diversified over approximately 12 months in the ICU.

Conclusions
This ICU's CRAB population was sustained through regular introductions of distinct clusters. Some GC2 clusters and a novel ST164 strain persisted for months, which suggests they were particularly well-suited for survival in ICU environments.

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