Young Microbiologists Symposium

on Microbe Signalling, Organisation and Pathogenesis

Robert Ryan
Helge Dorfmueller
Delphine Caly

Apex City Quay Hotel
Dundee UK
29-30th June 2016
Young Microbiologists Symposium 2016
Programme

Wednesday 29th June

8:45 – 9:15  Arrival and Registration (with coffee and light breakfast)

9:15 – 9:30  Introduction and Welcome given by the chair of the meeting

9:30 – 10:15  FEMS Lecture by Prof. Ute Römling (Karolinska Institutet, SE)
“Serendipity in Science”

Session I = Gene regulation and intracellular signalling
Chair: Jacob Malone (John Innes Centre, UK)

10:15 – 10:40  Max Dow (University College Cork, IE)
“Structural and functional insights into the regulatory actions of HD-GYP domain proteins”

10:40 – 11:05  Jana Hiltner (University of Strathclyde, UK)
“The paralogous pyruvate kinases in Streptomyces coelicolor have distinct roles in growth and specialised metabolite production”

11:05 – 11:35  Coffee break

11:35 – 11:55  Lisa Bowman (Imperial College London, UK)
“Cyclic-di-AMP metabolism in Staphylococcus aureus – what makes, breaks and triggers its production”

11:55 – 12:15  Francesca D’Angelo (University Roma Tre, IT)
“Generation of synthetic cells interfacing with bacterial pathogens for innovative drug delivery approaches”

12:15 – 13:00  ASM Lecture by Prof. Scott Hultgren (Washington University, USA)
“The molecular imprint left by E. coli pathogenesis: a blueprint to design novel therapeutics for UTI”

13:00 – 15:00  Lunch and poster session I

Session II = Host-microbe interactions – pathogenesis and commensalism
Chair: Nicola Stanley-Wall (University of Dundee, UK)

15:00 – 15:25  Marvin Whiteley (University of Texas, USA)
“The role of biogeography in polymicrobial infection”

15:25 – 15:50  Andrew Roe (University of Glasgow, UK)
“LOVe is the answer: new tools for studying host-pathogen interactions”

15:50 – 16:10  Dan Stones (University of Birmingham, UK)
“Targeting bacterial adherence suppresses burn wound infection with drug-resistant Pseudomonas aeruginosa”
16:10 – 16:40 Coffee break

16:40 – 17:00 Tuuli Ahlstrand (University of Turku, FI)  
“Intrinsically disordered bacterial outer membrane protein plays role in the response of an oral pathogen to cytokines”

17:00 – 17:20 Heather Hulme (University of Glasgow, UK)  
“Biomarker discovery during in vivo Salmonella Typhimurium infection through mass spectrometry imaging”

17:20 – 17:40 James Connolly (University of Glasgow, UK)  
“When and where – Pathogenic Escherichia coli differentially sense D-serine using a universal transporter system to specify their environment within the host”

19:00 – 21:00 Guest speakers’ dinner (by invitation only)

Thursday 30th June

8:30 – 9:00 Coffee and light breakfast

9:00 Introduction given by the co-chair of the meeting

Session III = Structure, biogenesis and transport across membranes  
Chair: Johann Habersetzer (University of Dundee, UK)

9:00 – 9:25 Alain Filloux (Imperial College London, UK)  
“The bacterial type VI secretion system: killing with moderation”

9:25 – 9:50 Emma Denham (University of Warwick, UK)  
“Deciphering the role sRNAs play in the different lifestyles of Bacillus subtilis”

9:50 – 10:10 Tessa Quax (University of Freiburg, DE)  
“Motility in Archea”

10:10 – 10:30 Francesca Cianfanelli (University of Dundee, UK)  
“VgrG and PAAR proteins define distinct versions of a functional Type VI secretion system”

10:30 – 11:00 Coffee break

Session IV = Poster talks and Workshop  
Chair: Delphine Caly (Lille1 University, FR)

11:00 – 13:00 “Hot-spot” poster talks (Talks in yet to be determined order)

Chinwe Chukwudi (University of Nigeria, NG)  
“Innovative use of the tetracyclines to activate bacterial suicide genes and combat antibiotic resistance mechanisms”

Daniel Pérez-Mendoza (University of Kassel, DE)  
“C-di-GMP binding domain involved in production a mixed-linkage β-Glucan in Sinorhizobium meliloti”
Fang-Fang Wang (Chinese Academy of Sciences, CN)
“Receptor histidine kinase directly binds plant chemical to promote bacterial adaptation in host plant”

Henry Schreiber (Washington University, USA)
“Interplay between dynamic bacterial virulence phenotypes of E. coli and host susceptibility determines UTI risk”

Lucia Grenga (John Innes Centre & University of East Anglia, UK)
“Adaptive remodeling of the bacterial proteome by specific ribosomal modification regulates Pseudomonas infection and niche colonisation”

Olivier Lesouhaitier (University of Rouen, FR)
“The lung hormone C-type natriuretic peptide (CNP) modifies Pseudomonas aeruginosa virulence and biofilm formation after binding to the AmiC bacterial protein: Mechanism of action”

Pauline Basso (Université de Grenoble, FR)
“Pseudomonas aeruginosa pore-forming Exolysin and type IV pili cooperate to induce host-cell lysis”

Richard Bauer (University Hospital Ulm, DE)
“CcpA as regulator of Streptolysin S in Streptococcus anginosus”

Simon Bo Lassen (Aarhus University, DK)
“Antimicrobial activity of human skin associated Staphylococcus epidermidis”

Stephania Spanò (University of Aberdeen, UK)
“Salmonella neutralizes a host defense pathway with a one-two punch”

OR
11:00 – 13:00 PLOS Pathogens Writing and Publishing Workshop
Neil Mabbott (The Roslin Institute and University of Edinburgh, UK)

13:00 – 14:20 Lunch and poster session II

14:20 – 15:20 EMBO Keynote Lecture by Prof. Christoph Tang (University of Oxford, UK)
“Fever pitch for bacterial pathogens”

Session V = Microbe – microbe interactions – pathogenesis and commensalism
Chair: Stephania Spanò (University of Aberdeen, UK)

15:20 – 15:45 Vanessa Sperandio (UT Southwestern Medical Center, USA)
“Enterohemorrhagic E. coli (EHEC) sings: pour some sugar on me!”

15:45 – 16:10 Beckie Ingram (Queens College Belfast, UK)
“Bacterial interactions within the lung”

16:10 – 16:30 Coffee break
16:30 – 16:50  **Clare Kirkpatrick** (University of Geneva, CH)
“Growth control switch by a DNA damage-inducible toxin-antitoxin system in *Caulobacter crescentus*”

16:50 – 17:10  **Ding Yichen** (Singapore Centre for Environmental Life Sciences Engineering, SG)
“NDM-producing Enterobacteriaceae could protect *Pseudomonas aeruginosa* from antibiotic treatment in a dual species biofilm model”

17:10 – 17:30  Closing remarks by **Prof. Tracy Palmer** (University of Dundee, UK)
Award ceremony

19:30  **Social dinner and Ceilidh (Apex hotel)**
Presentation of invited speakers
Dr. Ute Römling is Professor of Medical Microbial Physiology at Karolinska Institutet in Stockholm, Sweden. Her research interests include the regulation of cyclic di-GMP in morphotype expression in Enterobacteriaceae multicellular behaviour (biofilm formation), the role of biofilm extracellular matrix components on bacterial-host interactions, and *Pseudomonas aeruginosa* adaptation in the cystic fibrosis (CF) lung by the analysis of genetic variations, comparative protein expression, and the development of a biofilm formation model.

**Selected publications:**

- Dissecting the cyclic di-guanylate monophosphate signalling network regulating motility in *Salmonella enterica* serovar Typhimurium.  
  *Le Guyon S, Simm R, Rehn M, Römling U*  
  *Environ. Microbiol.* 2015

- A novel protein quality control mechanism contributes to heat shock resistance of worldwide-distributed *Pseudomonas aeruginosa* clone C strains.  
  *Environ. Microbiol.* 2015
Dr. Max Dow is a Lecturer at BIOMERIT Research Centre, School of Microbiology at University College Cork, Ireland. His research interest is to understand the role of the bacterial second messenger, cyclic di-GMP, on the regulation and synthesis of virulence factors in bacterial pathogens of plants and animals.

**Selected publications:**

- A systematic analysis of the role of GGDEF-EAL domain proteins in virulence and motility in Xanthomonas oryzae pv. oryzicola.
  
  
  *Scientific Reports.* 2016

- Functional and genomic insights into the pathogenesis of Burkholderia species to rice.
  
  
Scott Hultgren (Washington University, USA)

“The molecular imprint left by *E. coli* pathogenesis: a blue-print to design novel therapeutics for UTI”

Dr. Scott J. Hultgren is Professor of Molecular Microbiology and Director of the Centre for Women’s Infectious Diseases Research at Washington University in St. Louis, USA. His primary research focuses on understanding the mechanisms of *Escherichia coli* urinary tract infections ranging from the study of bacterial genetic/genomics and bacterial virulence factors (extracellular fibres and amyloid adhesin biogenesis), to targeted bacterial pilus, for the development of new therapeutics to combat acute or recurrent urinary tract infections.

**Selected publications:**

- Metabolic requirements of *Escherichia coli* in intracellular bacterial communities during urinary tract infection pathogenesis.  
  Conover MS, Hadjifrangiskou M, Palermo JJ, Hibbing ME, Dodson KW, and Hultgren SJ  
  *MBio*. 2016

- Structure of a Chaperone-Usher Pilus Reveals the Molecular Basis of Rod Uncoiling.  
  *Cell*. 2015
Dr. Marvin Whiteley is Professor of Molecular Biosciences at Dell Medical School and Director of the Centre for Infectious Disease at the University of Texas, USA. His group is interested in the bacterial social behaviour, particularly how bacteria communicate, how polymicrobial interactions impact physiology and virulence, how bacteria adapt to the host, and if the nutrient availability at the site of infection influences bacterial virulence.

**Selected publications:**

- Essential genome of *Pseudomonas aeruginosa* in cystic fibrosis sputum.  
  Turner KH, Wessel AK, Palmer GC, Murray JL, Whiteley M.  
  *Proc Natl Acad Sci U S A.* 2015

- Show me the SNPs. How bacterial sex generates diversity in the cystic fibrosis lung.  
  Darch SE, Whiteley M.  
  *Am J Respir Crit Care Med.* 2015
Dr. Andrew Roe is a Senior Lecturer in Bacteriology, Institute of Infection, Immunity and Inflammation, at the University of Glasgow, UK. His research uses *Escherichia coli* O157: H7 as a model organism to study the regulation and expression of virulence factors with respect to how *E. coli* colonise specific niches within the host. He is also interested in developing inhibitors of virulence factors, and the use of LOV-based fluorescent reporters to monitor protein production.

**Selected publications:**

- Visualizing translocation and localization of bacterial type III effector proteins using a genetically encoded reporter system.  
  *Applied and Environmental Microbiology.* 2016

- A highly conserved bacterial d-serine uptake system links host metabolism and virulence.  
  **Connolly, J. P. R., Gabrielsen, M., Goldstone, R. J., Grinter, R., Wang, D., Cogdell, R. J., Walker, D., Smith, D. G. E., and Roe, A. J.**  
Alain Filloux (Imperial College London, UK)

“The bacterial type VI secretion system: killing with moderation”

Dr. Alain Filloux is Professor in the Department of Life Sciences and also the chair of Molecular Microbiology at Imperial College London, UK. His research aims to tackle the problem of persistent, chronic infections by *Pseudomonas aeruginosa*. His group focuses on two essential molecular mechanisms of *P. aeruginosa*: biofilm formation and type VI effector protein secretion that are both co-regulated and highly associated with *P. aeruginosa* pathogenesis. Research approaches such as molecular microbiology, genetics, cellular microbiology, structural biology and biochemistry are used in his laboratory.

**Selected publications:**


Dr. Emma Denham is Assistant Professor in Microbiology and Infection at the University of Warwick, UK. Her research interest lies in how bacteria regulate their lifestyles through the use of non-coding RNAs (ncRNAs) which can be categorised as independent segments, antisense or untranslated regions and are condition-dependent. She uses *Bacillus subtilis* as the model organism to study how these ncRNAs function and to determine the target which they are regulating. Her group is also interested in investigating how bacteriocins kill their bacterial target species.

**Selected publications:**

- Small regulatory RNA-induced growth rate heterogeneity of *Bacillus subtilis*.

- The phosphoenolpyruvate : sugar phosphotransferase system is involved in sensitivity to the glucosylated bacteriocin sublancin.
Dr Tessa Quax is a Post-doctoral fellow in the Albers lab at the University of Freiburg, DE. Her research interest lies on the haloarchaeal archaellum and the study of motility in Archaea. Her group also focuses on the assembly of cell surface appendages in archaea and their role in adhesion and biofilm formation.

Abstract

The ability to sense and respond to external stimuli is a key feature of all living organisms. Motility is essential to achieve this goal. Archaea are able to swim using a rotating surface structure, the archaellum. It operates analogous to the bacterial flagellum, but its components are unrelated and are instead homologous to components of type IV pili. Therefore, the archaellum represents a unique motility structure, exclusively present in archaea. With a minimal set of proteins, it achieves rotational force based on ATP hydrolysis, instead of the proton motive force that drives the bacterial flagellum. In bacteria, transfer of external stimuli to the flagellum is mediated by the chemotaxis system, in which the response regulator CheY plays a central role by binding to the switch protein at the base of the flagellum. It is largely unknown how environmental signals are transferred to the archaeal motility structure. Some Archaea, such as Euryarchaea, have acquired chemotaxic components from bacteria via horizontal gene transfer. How the bacterial chemotaxis system was evolutionary adapted to function successfully in archaea remains a puzzle. We identified stimuli that activate rotation of the archaellum and aimed to understand the structural basis of interaction between the chemotaxis system and the archaeal motility machinery.

Selected publications:

- Codon bias as a means to fine-tune gene expression. 
  Quax TE, Claassens NJ, Söll D, van der Oost J 
  *Molecular Cell*. 2015

- Differential translation tunes uneven production of operon-encoded proteins.
  *Cell Reports*. 2013
Dr. Christoph Tang is Professor of Pathology at the University of Oxford, UK. His research interests focus on the investigation of the virulence mechanisms of *Neisseria meningitides*, *Shigella flexneri* and *Acinetobacter baumannii*. Through the understanding of the interactions between the host complement system and bacterial pathogen infections, his group is dedicated on devising approaches in vaccine development to prevent bacterial infections.

Selected publications:

- Nonfunctional variant 3 factor H binding proteins as meningococcal vaccine candidates. van der Veen S; Johnson S; Jongerius I; Malik T; Genovese A; Santini L; Staunton D; Ufret-Vincenty RL; Pickering MC; Lea SM; Tang CM. *Infect Immun*. 2014

- Temperature triggers immune evasion by Neisseria meningitidis. Loh E; Kugelberg E; Tracy A; Zhang Q; Gollan B; Ewles H; Chalmers R; Pelicic V; Tang CM. *Nature*. 2013
Vanessa Sperandio (UT Southwestern Medical Centre, USA)

“Enterohemorrhagic E. coli (EHEC) sings: pour some sugar on me!”

Dr. Vanessa Sperandio is Professor at the University of Texas, Southwestern Medical Centre, USA. Her research focuses on the quorum sensing (QS) regulation of virulence genes in enterohaemorrhagic Escherichia coli (EHEC) O157:H7 and how the bacterial QS signals interact with host signals during the infection of host epithelial cells. She believes that further understanding of this regulatory system will lead to the identification of additional virulence factors and provide novel targets for vaccine and drug development.

Selected publications:
- QseC mediates Salmonella enterica serovar Typhimurium virulence in vitro and in vivo. Moreira, C.G., Weinshenker, D. and Sperandio, V. Infection and Immunity. 2010
Beckie Ingram (Queen’s University, Belfast, UK)

“Bacterial interactions within the lung”

Dr. Beckie Ingram is a Senior Lecturer at the School of Medicine, Dentistry and Biomedical Sciences at Queen’s University, Belfast, UK. Her research interest is to understand the lymphocytic response to bacterial pulmonary infections in order to facilitate the rational design of vaccines. In particular her group focuses on how the innate and adaptive immune system interact during acute bacterial (Streptococcus and Pseudomonas) infections.

Selected publications:

- Innate Lymphoid Cells Are the Predominant Source of IL-17A during the Early Pathogenesis of Acute Respiratory Distress Syndrome. 

Abstracts of talks
The paralogous pyruvate kinases in *Streptomyces coelicolor* have distinct roles in growth and specialised metabolite production

JK Hiltner¹, P Cruz-Morales², L T Fernandez-Martinez³, H Petkovic⁴, IS Hunter¹, JF Barona-Gómez², PA Hoskisson¹

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3 John Innes Centre, Norwich
4 Acies Bio Ltd, Ljubljana, Slovenia

Streptomyces species are prolific producers of bioactive metabolites, nevertheless analysis of complete genomes still shows that there are many biosynthetic clusters present that are silent under normal cultivation conditions. The current increase in clinical antibiotic resistance requires the discovery of new antibiotics, but also a greater understanding of antibiotic production for industrial exploitation. Our interest is in studying the interaction of primary metabolites into specialised metabolism. We focus on the Phosphoenolpyruvate-Pyruvate-Oxaloacetate node of central carbon metabolism using *Streptomyces coelicolor* as a model and we have identified pyruvate kinase as a potential target that influences the production of antibiotics. The genome encodes two paralogue pyruvate kinase genes - SCO2014 (pyk1) and SCO5423 (pyk2). Phenotypic analysis of the two mutants revealed differences in their physiological role, pyk2 exhibits altered growth on glucose, whereas pyk1 mutants shows altered actin or hodin production. We used cross-species complementation experiments with the *E. coli* pyruvate kinase mutants ΔpykF, ΔpykA and ΔpykAΔpykF double mutant and complemented these with pyk1 and pyk2 from S. coelicolor on different media to clarify the physiological role. Furthermore Pyk1 and Pyk2 were overexpressed in *E. coli* and studied on their biochemical properties. The results indicate that Pyk2 is the house-keeping enzyme whereas Pyk1 is activated under low energy state condition as gluconeogenesis for example, as it requires AMP for activity. Our data show that paralogous genes in primary metabolism have distinct physiological roles in Streptomyces that impact significantly on growth and the production of antibiotics, which could be used for industrial strain improvement in the biotechnology industry.
Cyclic-di-AMP metabolism in *Staphylococcus aureus* – what makes, breaks and triggers its production

Lisa Bowman, Volkhard Kaever and Angelika Gründling

Section of Microbiology and MRC Centre for Molecular Bacteriology and Infection, Imperial College London, London, UK

Signalling nucleotides are key molecules in all domains of life and control fundamental processes including carbon metabolism and biofilm formation. Cyclic-di-AMP is one of the more recently discovered signalling molecules that is produced by a number of bacteria including pathogens such as *Staphylococcus aureus*. c-di-AMP has since been implicated in controlling cell size in *S. aureus*, in helping to cope with cell wall stress and in the regulation of potassium uptake. In this microorganism, c-di-AMP is synthesised from two molecules of ATP by the diadenylate cyclase DacA, and degraded to pApA by the phosphodiesterase GdpP. Currently, little is known about how DacA and GdpP expression is regulated in *S. aureus*, or the signals and environmental conditions that lead to changes in c-di-AMP levels. Here, we investigated the role of a second predicted phosphodiesterase Pde2 in *S. aureus*. Biochemical analyses and LC-MS/MS nucleotide measurements revealed that this enzyme can hydrolyse c-di-AMP, however its main physiological function was found to be the degradation of pApA to AMP. Another protein predicted to be involved in altering cellular c-di-AMP levels is YbbR, a membrane protein encoded in an operon with *dacA*. As part of this study, we constructed a *ybbR* mutant *S. aureus* strain. The growth of this mutant was not affected under standard laboratory conditions; however the mutant showed increased susceptibility to acid stress. On-going work is focussed on elucidating the mechanism behind this.
Generation of synthetic cells interfacing with bacterial pathogens for innovative drug delivery approaches

Francesca D'Angelo¹, Alessandro Zennaro¹, Marco Messina¹, Daniela Tofani¹, Yutetsu Kuruma², Livia Leoni¹, Pasquale Stano¹, and Giordano Rampioni¹

¹ Department of Science, University Roma Tre, Rome, Italy
² Department of Medical Genome Sciences, University of Tokyo, Chiba, Japan

Liposomes are cell-like micro-compartments already used in humans as drug-carriers. We envisage the generation of liposome-based synthetic minimal cells (SMCs) able to monitor their environment and to release or synthesize antimicrobials only in response to bacterial pathogens. To reach this goal, a proof-of-concept is required showing the possibility of generating SMCs able to interact with natural cells. Here we describe the generation of SMCs able to establish a “synthetic-to-natural” communication channel with the human pathogen *Pseudomonas aeruginosa*. Briefly, a protocol for reproducible generation of large amounts of SMCs has been set-up. This protocol has been used to generate SMCs containing i) a plasmid for the expression of RhlI, a synthase that catalyse the production of the quorum sensing signal molecules N-butyryl-homoserine lactone (C4-HSL), ii) the substrates of RhlI, and iii) the PURE system®, a recently developed cell-free transcription-translation kit containing the minimal amount of purified components for in vitro protein expression from a DNA template. By means of biochemical and analytical chemistry methods, we demonstrated that these SMCs are able to produce the *rhlI* RNA, to express the RhlI protein, and to synthesize the signal molecule C4-HSL. As expected, these SMCs are able to alter *P. aeruginosa* gene expression in a similar way as natural bacterial cells producing C4-HSL. Moreover, modification of the incubation medium allowed developing a “co-culture” system in which SMCs and *P. aeruginosa* can co-exist. The development of SMCs able to respond to signal molecule produced by *P. aeruginosa* is in course in our laboratory. The establishment of a “natural-to-synthetic” communication channel will pave the way for the generation of SMCs endowed with cognitive capacity, to be used as soft nanorobots for future intelligent drug delivery approaches.
Targeting bacterial adherence suppresses burn wound infection with drug-resistant *Pseudomonas aeruginosa*

Daniel Stones
Anne-Marie Krachler lab, Birmingham University

The rise of antibiotic resistant bacteria, together with the narrowing pipeline of newly developed antimicrobials makes it increasingly challenging to treat infections. Drugs targeting bacterial virulence instead of proliferation, and exert no selective pressure on antimicrobial resistance, are urgently needed. Adhesion is a key step in the early stages of infection of the host by bacteria and is required for delivery of virulence factors and survival. Previous studies have demonstrated that Multivalent Adhesion Molecules (MAMs), which consist of multiple mammalian cell entry (MCE) domains, enable a wide range of Gram-negative bacteria to establish high affinity binding to host cells at the early stages of infection. We found that an inhibitor, consisting of bead-coupled recombinant MAM7, prevents bacterial adherence to tissues, thereby suppressing infection with multidrug-resistant *Pseudomonas aeruginosa* in a rat burn and excision model. The inhibitor decreased bacterial loads in the wound and prevented the spread of the infection into adjacent tissues. Application of the inhibitor did not alter local and systemic inflammatory responses or wound healing. In addition, biochemical and structural analysis of MAMs from different Gram-negative bacteria has also highlighted key aspects of MAMs binding to host cells. Together, these results highlight an attractive novel strategy for the prevention and treatment of a wide range of bacterial infections, without leading to the emergence of drug-resistance.
Intrinsically disordered bacterial outer membrane protein plays role in the response of an oral pathogen to cytokines

Tuuli Ahlstrand1, Heidi Tuominen1,2, Perttu Permi3,4, Riikka Ihalin1

1 University of Turku, Turku, Finland
2 Biovian Oy, Turku, Finland
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Periodontitis, an inflammatory oral disease, is strongly associated with gram-negative opportunistic pathogen *Aggregatibacter actinomycetemcomitans*, which resides in the biofilm indento-gingival interphase. Besides having other virulence mechanisms, *A. actinomycetemcomitans* binds and uptakes human proinflammatory cytokine interleukin (IL)-1β. Our research group has discovered an outer membrane protein of *A. actinomycetemcomitans*, bacterial IL receptor I (BilRI), which binds IL-1β. Cytokine uptake may increase the bacterial virulence as it decreases the metabolic activity of bacteria within the biofilm and enhances the biofilm formation. It may also alter the biofilm composition and especially the amount of biofilm matrix components: extracellular proteins, DNA (eDNA) and polysaccharides. The NMR studies showed that BilRI does not naturally make a stable three-dimensional fold hence being an intrinsically disordered protein (IDP). Further experiments with BilRI revealed that it bound various cytokines (IL-8, IL-10, IFN-γ and TNF-α), except IL-6, with low affinity in microplate assay. The uptake of IL-8 and IL-6 was demonstrated in *A. actinomycetemcomitans* which were incubated with epithelial cells using immunoEM. However, BilRI only had a significant effect on IL-1β uptake in this test system. When biofilms were grown in the presence of 10 ng/ml of IL-1β or IL-8, cytokines significantly decreased the eDNA and polysaccharide amount in wild type *A. actinomycetemcomitans* biofilm but not in *bilRI* mutant strain. In the biofilms the protein amount did not change in response to cytokines. Broad binding capacity to different cytokines is probably due to the undefined folding of BilRI. The role of BilRI might be to concentrate cytokines on the cell membrane and its function maybe significant only when cytokines are present in low concentrations. The actual mechanism that transfers cytokines inside the bacterial cells is still unknown. Furthermore, the cytokine induced changes in biofilm composition need further characterization. The significance of this bacterial mechanism is eminent as it seems both to affect the virulence of the bacterium and have wider potential to interfere with the host defence by binding an array of cytokines.
Biomarker discovery during *in vivo* *Salmonella* Typhimurium infection through mass spectrometry imaging

Heather Hulme¹, Lynsey Meikle¹, Nicole Strittmatter², Hannah Wessel¹, John Swales², Anna Nilsson³, Richard Burchmore¹, Richard Goodwin², Donal Wall¹

¹ University of Glasgow, Glasgow, UK
² AstraZeneca, Cambridge, UK
³ Uppsala University, Uppsala, Sweden

*Salmonella enterica* serovar Typhimurium is a facultative intracellular bacterium which causes self-limiting gastroenteritis in human. The bacteria are transmitted by ingestion of contaminated food or water and infect cells of the small intestine. Once across the epithelial layer *Salmonella* are transported to the underlying mesenteric lymph nodes (MLNs). This is an important site in the immune response to the infection and *Salmonella* use many unique methods to disrupt the cells and activation of the immune system at this site. We utilised mass spectrometry imaging to find biomarkers of infection in the MLNs from an *in vivo* *Salmonella* colitis model to further understand the host pathogen interaction. One of the biomarkers discovered was particularly interesting as it localised to areas of bacterial infection and tissue disruption in the MLNs. The biomarker localised to areas of disrupted immune cells in the MLNs and this molecule was found to kill CD4+ T cells, therefore *Salmonella* could be causing the increased release of this biomarker by the host as a method to disrupt the immune system.
When and where - Pathogenic *Escherichia coli* differentially sense D-serine using a universal transporter system to specify their environment within the host

James PR Connolly

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Responding to the environment is critically important for bacteria looking to colonize a specific niche within the host. Furthermore, pathogenic bacteria can utilize site-specific signals to directly regulate the expression of their unique virulence genes thus giving them a competitive advantage. One such signal, D-serine, plays an important role in this process. D-serine is found in abundance in the urinary tract but much less so in the gut. Accordingly, uropathogenic *E. coli* (UPEC) carry a D-serine tolerance locus (*dsdCXA*) and can both catabolize it as a carbon source as well as respond to it transcriptionally. Enterohaemorrhagic *E. coli* (EHEC) on the other hand are mutated for the *dsdCXA* locus and this metabolite causes both stress and transcriptional repression of their type 3 secretion system (T3SS) used to intimately attach to the colonic epithelium. Strikingly, this reciprocal carriage of either the T3SS or *dsdCXA* is widespread across the entire *E. coli* phylogeny and suggests an evolutionary pressure for EHEC to colonize sites lacking in D-serine\(^1\). Recently, we have discovered that *E. coli* carries a second functional D-serine uptake system. The genes identified are highly conserved in all *E. coli* but are regulated differentially in unique pathogenic backgrounds. EHEC, counter-intuitively, increase D-serine uptake through this system upon encountering it in the environment, whereas UPEC do not. It was also found that the system has been integrated into the transcriptional network of the T3SS and is required for full virulence demonstrating an important pathotype-specific adaptation of this novel D-serine uptake system\(^2\). This work further highlights the relevance and complex nature of niche recognition for bacteria.

\(^1\) Connolly et al. (2015) ISME J (doi: 10.1038/ismej.2014.242. pmid:25526369)

\(^2\) Connolly et al. (2016) PLoS Pathog (doi:10.1371/journal.ppat.1005359)
VgrG and PAAR proteins define distinct versions of a functional Type VI secretion system

Cianfanelli F.R., Trost M. and S. J. Coulthurst

The Type VI secretion system (T6SS) is a macromolecular machinery, widespread in Gram-negative bacteria, which is used to deliver toxic proteins ("effectors") into target eukaryotic or prokaryotic cells. As a result, this system plays a crucial role both in direct pathogenicity and in interbacterial interactions, including polymicrobial infections. T6SS are encoded within large gene clusters containing, at least, fourteen conserved core components. These form a large trans-envelope machinery including an expelled puncturing device consisting of a tube formed by hexameric rings of Hcp, topped by a VgrG trimer and further sharpened by a PAAR-repeat protein. Anti-bacterial T6SS can deliver a variety of effectors targeting different compartments of the recipient cell, such as the peptidoglycan cell wall, the cell membrane and nucleic acid. Current models suggest that a variety of effectors can be injected in one single lethal shot, each either non-covalently associated or directly fused with one of the proteins forming the puncturing device. Serratia marcescens is an opportunistic pathogen that possesses a potent anti-bacterial T6SS. This T6SS is encoded by a large cluster in addition to several other loci encoding non-essential components and multiple effectors. Two different VgrG homologues and three PAAR-repeat proteins are encoded in the genome of S. marcescens Db10. Here, we used a combination of genetic, proteomic and phenotypic approaches to determine the role of the two VgrG and the three PAAR-repeat proteins in the functionality of the T6SS. We identified specific VgrG-PAAR combinations able to mediate active secretion and displaying different anti-bacterial activity, and we further showed that this latter difference could be due to delivery of specific subsets of antibacterial effectors.
Growth control switch by a DNA damage-inducible toxin-antitoxin system in *Caulobacter crescentus*

Clare Kirkpatrick

University of Geneva

Bacterial toxin-antitoxin (TA) systems are thought to respond to various stresses, often inducing growth-arrested (persistent) sub-populations of cells whose housekeeping functions are inhibited. Many such TA systems induce this effect through translation-dependent RNA cleavage (RNase) activity of their toxins, which are held in check by their cognate antitoxins in the absence of stress. However, it is not always clear whether specific mRNA targets of orthologous RNase toxins are responsible for their phenotypic effect, which has made it difficult to accurately place the multitude of TA systems within cellular and adaptive regulatory networks. Here we show that the TA system HigBA of *Caulobacter crescentus* can promote and inhibit bacterial growth dependent on the dosage of HigB, a toxin regulated by the DNA damage (SOS) repressor LexA in addition to its antitoxin HigA, and the target selectivity of HigB’s mRNA cleavage activity. At low expression, HigB reduced the expression of an efflux pump that is toxic to a polarity control mutant, improving its growth. By contrast, it acts as an inducible cell death mechanism in cells lacking LexA or exposed to DNA damage (which causes high-level toxin expression) and targets the cell cycle circuitry through the master cell cycle regulator CtrA. Thus, TA systems can have outcome switching activity in bacterial adaptive (stress) and systemic (cell cycle) networks.

Kirkpatrick CL et al, 2016, Nature Microbiology, doi:10.1038/NMICROBIOL.2016.8
NDM-producing Enterobacteriaceae could protect *P. aeruginosa* from antibiotic treatment in a dual species biofilm model

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Resistance to antibiotics is recognized as one of the major global threats of the 21st century. The abuse of antibiotic use in the past decades has led to prevalence of multiple drug resistant microorganisms throughout the world. New Delhi metallo-β-lactamase-1 (NDM-1) was recently identified in 2009. It has a potent hydrolytic activity against almost all carbapenems, which are the last resort β-lactam to treat infections caused by extended-spectrum β-lactamase producing bacteria. Since its discovery, NDM-1 producing Enterobacteriaceae has been spreading worldwide at an alarming rate. Various studies have been done to investigate the genetics, epidemiology and antimicrobial resistance of NDM-1 positive bacteria. Here, we showed that three NDM-1 producing Enterobacteriaceae species, namely *E. coli*, *Klebsiella pneumoniae*, and *Citrobacter amalonaticus* could protect the opportunistic pathogen *Pseudomonas aeruginosa* from carbenicillin. More specifically, we found that *P. aeruginosa* could form dual-species microcolonies with NDM-1 producing *C. amalonaticus* in the presence of carbenicillin. The ability to form dual-species biofilm with *P. aeruginosa* might explain the better protective effect of *C. amalonaticus* on *P. aeruginosa* against carbenicillin compared to NDM-1 producing *E. coli* and *K. pneumoniae*. We also showed that the exopolysaccharide Pel and flagellum-mediated swimming motility are essential for *P. aeruginosa* to form microcolonies in our dual-species biofilm model. Our results suggested that the *P. aeruginosa* can utilize the carbenicillin resistance of NDM-1 producing strains, which could be a novel mechanism of antimicrobial resistance.
“Hot-spot” poster talks
Salmonella neutralizes a host defense pathway with a one-two punch

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Host defense mechanisms protect animals against the attack of microorganisms, including invasive bacteria. We identified a host trafficking pathway that prevents the human-restricted pathogen *Salmonella Typhi* from surviving in mouse macrophages and therefore infecting mice. This pathway depends on the host GTPase Rab32 and its guanine nucleotide exchange factor BLOC-3. The potential broader role of this pathway in host defense is unknown. We report that the Rab32/BLOC-3 pathway is neutralized by two type-III-secretion effectors delivered by the broad host pathogen *Salmonella Typhimurium*. In addition to GtgE, which is a specific protease cleaving Rab32, *Salmonella Typhimurium* delivers SopD2, which is a Rab GTPase activating protein (GAP) that inactivates Rab32. Each of these effectors is sufficient to confer *Salmonella Typhimurium* the ability to infect mice. A *Salmonella Typhimurium* strain deficient for both these effectors is unable to infect mice, yet it is fully virulent in BLOC-3 deficient mice. These results indicate that the *Salmonella Typhimurium* effectors GtgE and SopD2 act redundantly to neutralize a powerful host defense pathway that can prevent *Salmonella* infections in mice. Interestingly, the Rab32/BLOC-3 host defense pathway does not require known antimicrobial mechanisms to clear *Salmonella* infections suggesting that it functions in conjunctions with novel antimicrobial factors.
CcpA as regulator of Streptolysin S in *Streptococcus anginosus*

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*Streptococcus anginosus*, as a member of the *Streptococcus anginosus* group, is a commensal of mucosal membranes, but also an emerging human pathogen. Some *S. anginosus* strains, including the type strain, show a prominent β-hemolytic phenotype. The genetic locus (*sag* gene cluster) of the hemolysin expression has recently been described and it was shown that this virulence factor exhibits high homologies with the Streptolysin S (SLS) of *Streptococcus pyogenes*. It was observed that the hemolytic activity as well as the expression of the *sag* gene cluster was reduced in the presence of high glucose concentrations. The expression of the hemolysin was thereby investigated in a promoter reporter assay in which the expression of EGFP is driven by the *sag* promoter. However, regulation mechanisms controlling the expression of the SLS in *S. anginosus* have not been elucidated so far. The use of different sugars in the reporter assay lead to the hypothesis that carbon catabolite repression (CCR), a well investigated regulation mechanism in Gram+ bacteria controls hemolysin expression. A key player in CCR is the regulator called Catabolite control protein A (CcpA). In *S. pyogenes* CcpA regulates the expression of the SLS, therefore, the role of CcpA in expression of the *sag* gene cluster of *S. anginosus* was investigated. A CcpA insertion mutant was constructed, transformed with the reporter plasmid carrying the *sag* promoter in front of EGFP and the activity of the promoter was determined in dependency to the glucose concentration of the medium. The previously described reduction of gene expression in the presence of high glucose concentration in the wild type reporter strain is abolished in the CcpA mutant demonstrating that CcpA is involved in the regulation of the *sag* gene cluster. Based on this observation the promoter region of the *sag* gene cluster was analysed bioinformatically to identify putative CcpA binding sites. This resulted in three potential CcpA binding sites which were tested in the promoter reporter assay by using site directed mutagenesis. The 3 putative binding sites were separately mutated or deleted and cloned in front of EGFP in the reporter plasmid. Promoters that contain a mutation in the CcpA binding site should not show a glucose dependent reduction of activity. With this assay a potential CcpA binding site, with good homologies to the consensus binding site of CcpA, could be identified. The expression data could be verified in a functional hemolysis assay in which the CcpA mutant was able to lyse human erythrocytes in the presence of high glucose concentrations. In summary this study characterized for the first time a regulator of the emerging pathogen *S. anginosus*. We could demonstrate that CcpA is involved in the regulation of SLS of *S. anginosus* and identified a putative CcpA binding motif in the SLS promoter region.
*Pseudomonas aeruginosa* pore-forming Exolysin and Type IV pili cooperate to induce host-cell lysis

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*Pseudomonas aeruginosa* clinical strains lacking Type III secretion system genes employ a novel toxin, Exolysin (ExlA), for host cell destruction. Here, we demonstrated that ExlA uses a predicted outer membrane protein ExlB for its export through two POTRA domains showing that ExlA-ExlB represent new members of a Two-Partner Secretion (TPS) systems. In addition of TPS secretion signals, ExlA harbors several distinct domains, which comprise notably hemagglutinin FHA domains, five integrin binding motifs Arginine-Glycine-Aspartic acid (RGD) and a non-conserved C-terminal region. Cytotoxic assays showed that the deletion of the C-terminal region abolishes host-cell cytolysis. Using red blood cells and lipid vesicles, we demonstrate that ExlA has a pore-forming activity that precedes cell membrane disruption in nucleated cells. Finally, by setting up a miniaturized cellular live-death assay, we screened a transposon mutant library of an ExlA-producing *P. aeruginosa* clinical strain for bacterial factors required for ExlA-dependent toxicity. The screen allowed the identification of proteins of Type IV pili as being absolutely required for ExlA-dependent cell destruction. This is the first example of cooperation between a TPS pore-forming toxin and surface appendages in host cell intoxication.
Innovative use of the tetracyclines to activate bacterial suicide genes and combat antibiotic resistance mechanisms

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The tetracyclines are a broad spectrum group of antibiotics whose antibacterial efficacy has been greatly reduced due to the development of resistance. However, their use in non-bacterial diseases continues to increase though their mechanism of action in these conditions is still poorly understood. Our recent studies indicate an effect on the secondary structures, processing and consequently, function of cellular RNAs under certain conditions. The \textit{hok/sok} locus is a well-established toxin/antitoxin plasmid stability element, which has been associated with multi-drug (ampicillin, chloramphenicol, kanamycin, streptomycin and sulphonamides) resistance plasmids. It is one of the most frequently reported plasmid addiction systems in ESBL-encoding plasmids, and ensures plasmid maintenance by post-segregational killing of plasmid-free daughter cells via RN:RNA interactions. The \textit{hok/sok} locus also occurs in the chromosomes of enterobacteria, and is more abundant in pathogenic strains. Our recent studies also indicate that the \textit{hok/sok} locus enhances host cell survival, especially in stressful conditions such as high temperature, antibiotic stress and low cell concentrations. Interestingly, we observed that although the \textit{hok/sok} locus increases bacterial tolerance to \ensuremath{\beta}-lactam antibiotics, it increases the susceptibility of these bacteria to the tetracyclines (doxycycline) by inducing the self-killing plasmid maintenance system due to the interference of the tetracyclines with the RNA:RNA processing pathway. Hence, the \textit{hok/sok} locus may represent a drug target that will open up opportunities for the innovative use of tetracyclines in the global war to contain the rise of antimicrobial resistance.
Adaptive remodeling of the bacterial proteome by specific ribosomal modification regulates *Pseudomonas* infection and niche colonisation.

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Post-transcriptional control of protein abundance is a highly important, underexplored regulatory process by which organisms respond to their environments. Here we describe an important and previously unidentified regulatory pathway involving the ribosomal modification protein RimK, its regulator proteins RimA and RimB, and the widespread bacterial second messenger cyclic-di-GMP (cdG). Disruption of *rimK* affects motility and surface attachment in pathogenic and commensal Pseudomonas species, with *rimK* deletion significantly compromising rhizosphere colonisation by the commensal soil bacterium *P. fluorescens*, and plant infection by the pathogens *P. syringae* and *P. aeruginosa*. RimK functions as an ATP-dependent glutamyl ligase, adding glutamate residues to the C-terminus of ribosomal protein RpsF and inducing specific effects on both ribosome protein complement and function. Deletion of *rimK* in *P. fluorescens* leads to markedly reduced levels of multiple ribosomal proteins, and also of the key translational regulator Hfq. In turn, reduced Hfq levels induce specific downstream proteomic changes, with significant increases in multiple ABC transporters, stress response proteins and non-ribosomal peptide synthetases seen for both Δ*rimK* and Δ*hfq* mutants. The activity of RimK is itself controlled by interactions with RimA, RimB and cdG. We propose that control of RimK activity represents a novel regulatory mechanism that dynamically influences interactions between bacteria and their hosts; translating environmental pressures into dynamic ribosomal changes, and consequently to an adaptive remodeling of the bacterial proteome.
Antimicrobial activity of human skin associated *Staphylococcus epidermidis*

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The human skin is colonized by a complex mixture of bacteria, fungi and viruses. Some bacterial species can be predominant depending on the specific skin location, including the species *Staphylococcus epidermidis* and *Propionibacterium acnes*. The interference between these two species and its impact on skin health and disease is largely unknown. Interspecies competition can impact on the fine balance of the human skin ecosystem, which might initiate or support certain skin disorders such as acne vulgaris. In this study we investigate the antimicrobial activity of human skin-associated strains of *S. epidermidis*, aiming at the identification of antimicrobial mechanisms as executors of intercellular competition on human skin. Strains of *S. epidermidis* were screened for antimicrobial activity against *P. acnes*. Strains capable of inhibiting *P. acnes* were selected for genome sequencing and analysis. Genes encoding putative bacteriocins and other antimicrobial functions were identified. Preliminary results indicate that a group of *S. epidermidis* strains encodes a so far uncharacterized group of class II bacteriocins. Another strain, exhibiting an extraordinary antimicrobial activity against both *P. acnes* and other *S. epidermidis* strains, possesses an unusual type VII-like secretion system linked to a polymorphic toxin locus that might be involved in the antimicrobial activity. The antimicrobial activities of the tested strains have proven effective in inhibiting *P. acnes* and could potentially also inhibit other microorganisms. These strains or their active compounds could therefore pose a possible remedy against *P. acnes* associated diseases as well as against other opportunistic infections.
The lung hormone C-type natriuretic peptide (CNP) modifies *Pseudomonas aeruginosa* virulence and biofilm formation after binding to the AmiC bacterial protein: Mechanism of action

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There is now ample evidence that bacterial virulence is modulated by eukaryotic messengers including hormones. We have shown previously that the C-type Natriuretic Peptide (CNP), a peptide produced by lung, enhances *Pseudomonas* virulence. In the present work, we studied the effect of CNP on bacterial biofilm formation and we looked for a putative *P. aeruginosa* protein that could explain the effect of CNP on bacteria. We used *in silico* and *in vitro* approaches to identify a bacterial sensor for CNP. Additionally, pharmacological tools were used as an innovative strategy to characterize the binding site in *P. aeruginosa*. We observed that CNP strongly inhibits *P. aeruginosa* biofilm formation. This effect is totally prevented by Isatin, an antagonist of natriuretic peptide receptors in eukaryotic cells. The actions of CNP on *P. aeruginosa* virulence are mimicked by the eukaryotic receptor NPR-C agonist cANF4-23. Screening and comparing 3D structures of human natriuretic peptides receptors and *Pseudomonas* proteins revealed that the bacterial protein AmiC shows significant homology with the human C-type natriuretic natriuretic peptide receptor (hNPR-C). Furthermore, these analyses showed that both CNP and Isatin can interact with AmiC using the same amino acids as in hNPR-C. Finally, recombinant protein AmiC was purified and the protein interactions assessed using MicroScale Thermophoresis. The results showed that both CNP and an hNPR-C agonist bind AmiC protein with a KD of 2 µM and less than100 nM respectively, whereas hNPR-A agonist has poor affinity for AmiC. Using an amiC-mutant strain and its complemented derivative, we definitively validated the crucial role of AmiC protein on CNP’s effect on *P. aeruginosa* biofilm formation. As observed for hNPR-C in mammals, AmiC is highly selective and can discriminate between the different natriuretic peptides, since another natriuretic peptide (brain natriuretic peptide, BNP) has no affinity with AmiC. In conclusion, our work provides the first demonstration that the bacterial protein AmiC may be an ortholog of the eukaryotic receptor hNPR-C, acting as a CNP sensor in *P. aeruginosa*. AmiC appears to modulate a switch between chronic and acute infection phenotypes depending on exposure to host factors. The observation that CNP strongly decreases bacterial biofilm formation should have major consequences for cystic fibrosis treatment.

1 Lesouhaitier et al., 2009; Sensors 9: 6967-6990
2 Blier et al., 2011; Microbiology 157:1929-1944
An artificial increase of c-di-GMP levels in *Sinorhizobium meliloti* 8530 leads to overproduction of a mixed-linkage \((1 \rightarrow 3)(1 \rightarrow 4)\) \(\beta\)-d-glucan (MLG) that resembles ML \(\beta\)-glucans found in cereals and lichens\(^1\). In contrast to lichenan and barley glucans, this unique bacterial exopolysaccharide has a distinctive primary structure with a perfect alternation of \(\beta(1 \rightarrow 3)\) and \(\beta(1 \rightarrow 4)\) bounds, which may give new interesting biotech properties to this biopolymer\(^2\). Moreover, the MLG participates in bacterial aggregation and biofilm formation, and is required for efficient attachment to the roots of a host plant, resembling the biological role of cellulose in other plant associated bacteria\(^1\). A two-gene operon *bgsBA* required for production of this MLG, is conserved amongst several genera within the order Rhizobiales, where *bgsA* encodes a glycosyl transferase (GT) with domain resemblance and phylogenetic relationship to bacterial cellulose synthases (CS). Furthermore, MLG synthesis is also subjected to both transcriptional and posttranslational regulation, but in contrast to cellulose: (i) *bgsBA* transcription is dependent on the ExpR/SinI quorum sensing regulatory system and (ii) a novel c-di-GMP binding domain, different to PilZ, is involved in the posttranslational regulation\(^1\). Different Biomolecular Interaction Analysis (BIA), including Fluorescence Polarisation (FP) and Surface Plasmon Resonance (SPR)\(^3\), have been used in this study to characterise the binding of BgsA to c-di-GMP. Various site directed BgsA mutants has been assayed to identify the residues involved in the c-di-GMP binding and activation of MLG production. Preliminary results obtained so far suggest a different activation mechanism from other c-di-GMP-activated GTs like cellulose synthase.


Receptor histidine kinase directly binds plant chemical to promote bacterial adaptation in host plant

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How phytopathogenic bacteria recognize host plant derived chemical signals and elicit infection remains to be an opening question. In bacterial cells, two-component signal transduction system (TCS), consisting of a membrane-bound receptor histidine kinase (RHK) and a cytosolic response regulator, is the canonical sense-and-response machinery to react to various environmental stimuli via protein phosphorylation. Based on large-scale screening of ligand-receptor interactions, we demonstrated that PcrK-PcrR, a TCS of the plant black rot disease pathogen Xanthomonas campestris pv. campestris, directly senses an important plant chemical and modulates stress responses in host during pathogenesis. PcrK specifically binds the plant chemical with a high affinity, and modulate the phosphorylation level of PcrR. PcrR regulates about 60 genes in response to the plant chemical stimulation. Among them, an outer membrane protein was confirmed to play an important role in ROS stress response to the host environment. Our study revealed a case of inter-kingdom signalling transduction process, which will give insight into our understanding on the molecular coevolutionary mechanism during host-pathogen interactions.
Interplay between dynamic bacterial virulence phenotypes of *E. coli* and host susceptibility determines UTI risk

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Urinary tract infections (UTIs) are very common and most often caused by *Escherichia coli*. However, unlike many enteric *E. coli* pathogroups, no genetic signature has been identified for *E. coli* associated with cystitis. Towards this end, we used high-resolution comparative genomics to study 43 urine-associated *E. coli* (UAEC) strains isolated from 14 women suffering frequent recurrent UTIs. We found that these UAEC strains were genetically diverse, clustering into 21 distinct clonal groups that represented 4 major *E. coli* phylogenetic clades. Comparative genomic analysis of representative strains from each of these clonal group and 46 reference *E. coli* revealed that carriage of previously defined putative urovirulence factors correlated with phylogeny but not with a strain’s association with urinary disease. While UAEC strains were able to colonize several mouse models of acute cystitis significantly better than gut-associated *E. coli*, the urovirulence of UAEC strains varied significantly among different mouse strains and in single or competitive infections. No single set of genes in UAEC was predictive of bladder colonization in any of the mouse models. In contrast, comparative transcriptomic and phenotypic studies revealed significant variations in the expression of conserved functions and key behaviours among UAEC strains when grown under the same culture conditions, several of which were predictive of bladder colonization in C3H/HeN mice. These include variations in the expression of genes associated with motility and nutrient utilization and differences in both mannose-sensitive and mannose-resistant hemagglutination activity. Taken together, our findings suggest a new conceptual model of uropathogenesis in which UTI risk and outcome is determined by complex interactions of dynamic host susceptibility determinants and diverse bacterial urovirulence potentials that are driven not only by gene content but also by differences in the expression and regulation of conserved functions.