# BACPATH 15

## THE MOLECULAR BIOLOGY OF BACTERIAL PATHOGENS

The Australian Society for Microbiology

bringing Microbiologists together

30th Sept – 3rd Oct 2019 | The Novotel Vines Resort and Country Club, Swan Valley, WA

www.bacpath.org/ @BacPath #BacPath15

# CONTENTS

# WELCOME FROM THE CONFERENCE COMMITTEE

The BacPath conference has been an essential destination for Australian microbiologists undertaking investigations into the molecular biology of bacterial pathogens since 1991. Over the past 28 years, this conference has had tremendous support from this community which has enabled invitations to many of the world's leading experts to visit Australia and foster collaborative linkages in this research area. Along the way, the conference has rotated across most states of Australia except for Tasmania and Western Australia and has been held in some of the most picturesque locations each region has to offer. Our team in Western Australia are delighted to be able to continue this wonderful tradition, and for the first time, welcome our delegates to the Swan Valley in Western Australia. We are in the fortunate position to offer free tours of this exceptional wine and food region during your stay.

We are indebted to the industry representatives who have sponsored our conference this year. Please take the time to talk to their representatives from Millennium Science (Silver) and Melbourne University and Monash University (Bronze). We would like to thank our session sponsors for their ongoing support. We are also excited to announce for the first time, that the Australian Society for Microbiology (ASM) is our sponsor for the ASM BacPath Oration which recognises an outstanding contribution to the field of bacterial pathogenesis every two years. We are delighted that we have been able to invite two international speakers who have been sponsored by the Raine Visiting Professor Program at the University of Western Australia and the Perth Convention Bureau.

We have also received sponsorship from the American Society for Microbiology for a poster prize for a second year that is organised by Professor Melissa Brown. In addition, for the first time, we have attracted the support of the FEMS journal "Pathogens and Disease" for a grand oral presentation prize that has been organised by the co-editor Associate Professor Willa Huston.

Once again, this meeting would not be possible for the community support for the meeting, which has attracted over 160 delegates to make the trip to Western Australia.

Please enjoy our hospitality and the exceptional scientific program!

## BACPATH 15 ORGANISING COMMITTEE

### CHAIR

Associate Professor Charlene Kahler (University of Western Australia)

### COMMITTEE MEMBERS

Professor Geoff Coombs (Murdoch University)

Dr Mitali Sarkar Tyson (University of Western Australia)

Dr Josh Ramsay (Curtin University)

Dr Daniel Knight (Murdoch University)

Nicole Bzdyl (University of Western Australia)

# BACPATH 15 SPEAKERS

## Professor Tim Atkins

*Defence Science and Technology Laboratory, UK*

Prof. Tim Atkins is the Dstl Senior Fellow within the CBR division of the Defence Science and Technical Laboratory at Porton Down. Educated at Aberdeen University as an undergraduate he went on to complete a PhD at Imperial College London. Prof Atkins is the senior scientist with the division overseeing the technical content of the research programme. He is specifically responsible for the content of a programme of work to develop novel medical countermeasures (MCM) to biothreat agents and is the Lead Technical Reviewer for the majority of the programme. The biological MCM programme covers a range of technical specialisations including pre-treatments and therapies, bacterial and viral biothreat agents, high containment working and the development of animal models of infection. Prof Atkins' has expertise in research and development of medical countermeasures to biothreat agents and extensive linkages to external academic and industry groups working in this area. Previously, Prof Atkins has been seconded into MoD where he led a team of assessment staff. Currently, he holds a visiting position at Exeter University. He has sat as a member of committees ranging from fatal accident investigation to review of grant applications to charitable trusts.

## Dr Susanne Gebhard

*University of Bath, UK*

Susanne trained in molecular microbiology in Germany and New Zealand. Her PhD work was focused on transport systems and their regulation, while her first postdoctoral project saw her shift her research towards the regulation of antibiotic resistance. With her move back to Germany in 2009, Susanne combined these two interests and began to investigate how a transport system can play an active role in bacterial signalling. With this new project, she established an independent junior research group in Munich. In 2014, she moved to the UK to take up a lectureship in Medical Microbiology at the University of Bath, where she is continuing and expanding this work.

## Professor Paal Skytt Andersen

*Staten Serum Institute, Denmark*

Professor Skytt Andersen conducts research in the fields of genetic epidemiology, microbiomics, bioinformatics, and host-pathogen interactions. The primary focus of his research has been susceptibility to disease both infectious diseases (such as *Staphylococcus aureus* infections) and complex diseases such as inflammatory diseases (e.g. Inflammatory bowel disease and rheumatoid arthritis). This has involved the establishment of biobanks; human genome-wide association studies (GWAS) and bacterial GWAS in international collaborations including those with Prof. Vance Fowler, Duke University, NC, USA and Prof. Francois Vandenesch, University of Lyon, France. He is deeply involved in genomic epidemiology to better understand the evolution of virulent bacterial clones and antibiotic resistance. Dr Skytt Andersen also performs research within the field of microbiomics to understand microbial communities and their role in promoting or preventing disease (e.g. MRSA colonization, Atopic dermatitis; inflammatory bowel disease).

## Professor Ann Jerse

*Uniformed Services University, USA*

Dr Jerse has worked in the field of sexually transmitted infections since 1991 and developed the first small animal models of *Neisseria gonorrhoeae* (Gc) genital tract infection and Gc/chlamydial coinfection for studying pathogenesis in a whole model system and to accelerate product development. To help in the surveillance against antibiotic resistant Gc, Dr Jerse's laboratory also maintains a biorepository for Gc isolated at U.S. military treatment facilities and overseas sites. She also studies how compensatory evolution and fitness advantages conferred by certain antibiotic resistance alleles contribute to the spread of antibiotic resistant Gc. Translational research includes the development of gonorrhea vaccines and the pre-clinical testing of candidate antibiotics, vaccines, and vaginal microbicides against gonorrhea through agreements with NIAID or outside universities and pharmaceutical companies.

## Professor Richard Titball

*University of Exeter, UK*

Rick Titball is Professor of Molecular Microbiology at the University of Exeter. He moved to the University of Exeter from the Defence Science and Technology Laboratory (Dstl) at Porton Down in 2007, where he was a Senior Fellow. He has worked on bacterial pathogens of humans and animals including *Burkholderia pseudomallei*, *Yersinia pestis* and *Clostridium perfringens* and developed vaccines against plague and *C. perfringens* toxins which are now being exploited by industry. His work at Exeter is directed towards understanding the molecular basis of virulence of *B. pseudomallei* and *C. perfringens* and the molecular biology of toxin-antitoxin systems. At Exeter an underpinning feature of his research has been the use of alternative infection models, including *G. mellonella* larvae, to study disease. He has published over 300 peer-reviewed papers including work in Nature, Science and PNAS. He has 18 currently active patents. In 2015 he co-founded Biosystems Technology, a company developing alternatives to mammals for biological research development and testing. His work has been funded by the UK Dstl, BBSRC, MRC and Wellcome Trust, Innovate UK and NC3Rs and internationally by the Cariplo Foundation, US NIH and US DTRA.

# ASM BACPATH ORATION

Every BacPath conference has included a BacPath Oration to celebrate the contribution of a leading Australian scientist who has not only made an important contribution to our community in Australia but also has an outstanding international profile for their leadership in studying bacterial pathogens. It is our pleasure to announce that for the first time, this oration is now co-sponsored with the Australian Society of Microbiology and is now officially called the ASM BacPath Oration. This oration will be recognised as a national award similar to the ASM Bazeley and Snowdon Orations.

**2019 ASM BacPath Orator**

Professor Mark Schembri (University of Queensland)

Professor Mark Schembri is an NHMRC Senior Research Fellow at the University of Queensland. He is also Deputy Director of the Australian Infectious Diseases Research Centre. His research is in the field of molecular microbiology and bacterial pathogenesis. His specialist interest is in the area of uropathogenic *Escherichia coli* (UPEC), with a focus on the genetics, genomics and virulence of multidrug-resistant UPEC clones, and the role of cell-surface factors in UPEC adhesion, aggregation, biofilm formation and colonisation of the urinary tract. Professor Schembri has published >200 papers, including seminal research discoveries on the role of UPEC adhesins in disease and the evolution of the recently emerged and globally disseminated multidrug-resistant UPEC ST131 clone. His team have developed the application of transposon directed insertion-site sequencing (TraDIS) as a genome-wide screen to understand the genetic basis of complex UPEC phenotypes, including survival in human serum, capsule production, curli biosynthesis, antibiotic resistance, motility, plasmid conjugation and zinc resistance. In total, his research papers have been cited >13,000 times.

Professor Schembri obtained his PhD in 1996 from Monash University under the supervision of Prof John Davies. He then carried out a highly successful postdoc at the Technical University of Denmark under the mentorship of Prof Per Klemm, where he began his work on *E. coli* and the study of fimbrial and autotransporter adhesins. In 2001, he was awarded a highly prestigious 3-year Skou Fellowship from the Danish Science Foundation. Professor Schembri returned to Australia in 2004 to take up a Senior Lectureship position at the University of Queensland. He was awarded an ARC Future Fellowship in 2010 and his NHMRC SRF in 2016. Professor Schembri is also a Fellow of the Australian Society for Microbiology and an Elected Fellow of the American Academy of Microbiology. Professor Schembri's leadership in the field of UPEC research is evidenced by invitations to present the keynote talk at two out of the last three clinical urinary tract infection meetings (2013 and 2019). He has published 68 papers in the last 5 years, including a series of highlight 2019 publications in Nature Microbiology, Nature Communications (x2), PNAS, FASEB Journal and MBio.

# BACPATH PAST CONFERENCES AND INVITED SPEAKERS

| Year | Location | International | Australian |
|---|---|---|---|
| 1991 | Thredbo, NSW | Staffan Normark<br>Tony Roberts | Paul Manning |
| 1992 | Marysville, VIC | Roy Curtiss<br>Ben van der Zeijst | Roy Robins-Browne |
| 1995 | Marysville, VIC | Jorge Galan<br>Tony Pugsley | John Mattick |
| 1997 | Jamberoo, NSW | Chihiro Sasakawa<br>Mumatz Virji | Jim Pittard |
| 1999 | Victor Harbour, SA | Chris Raetz<br>Hans Wolf-Watz | Richard Alm |
| 2001 | Marysville, VIC | Bonnie Bassler<br>Mike Gilmore<br>Maggie So | Peter Reeves |
| 2003 | Jamberoo, NSW | Singh Chhatwal<br>Linda Kenny<br>George Weinstock | Julian Rood |
| 2005 | Couran Cove, QLD | Mike Apicella<br>Carol Gross<br>Paul Manning | Michael Good |
| 2007 | Lorne, VIC | Klaus Aktories<br>Carmen Buchrieser<br>Stephen Lory | Ben Adler |
| 2009 | Barossa Valley, SA | Laura Frost<br>Bob Hancock<br>Miguel Valvano | James Paton |
| 2011 | Kooindah Waters, NSW | Richard Brennan<br>Andrew Camilli<br>Gordon Dougan<br>Maria Schumacher | Mark Walker |
| 2013 | Tangalooma, QLD | Lauren Bakaletz<br>Scott Hultgren<br>Victor Nizet<br>Elaine Tuomanen | Michael Jennings |
| 2015 | Silverwaters, VIC | Ian Henderson<br>Jay Hinton<br>Meta Kuehn<br>Denise Monack | Elizabeth Hartland |
| 2017 | Adelaide Hills, SA | Tim Mitchell<br>Shelley Payne<br>William Shafer<br>Thomas Kehl-Fie<br>Jason Rosch | Ruth Hall |

# DELEGATE INFORMATION

## REGISTRATION DESK – ASN EVENTS

The registration desk is located in the foyer of the Novotel Vines Resort & Country Club. Any enquiries regarding your participation in BacPath 15 Conference can be directed to the ASN staff onsite. The registration desk opening hours are as follows:

- Monday 30th: 3:00 PM – 6:00 PM
- Tuesday 1st: 8:00 AM – 6:30 PM
- Wednesday 2nd: 8:00 AM – 6:30 PM
- Thursday 3rd: 8:00 AM – 12:40 PM

## REGISTRATION DETAILS

**Full Registration Entitlements**

- Entry to all sessions
- Lunch, morning and afternoon tea daily as outlined in the program
- Attendance at Poster Session on Monday and Tuesday, all included food and drinks
- Attendance at Tuesday evening dinner, all included food and drinks
- Attendance at the Wednesday ASM Oration Dinner at Sandalford Winery, all included 3-course meal and drinks, and return transport from the Vines to Sandalford.
- Access to the electronic abstracts
- Breakfast each day of the conference (breakfast for partners can be purchased for $32 per person)

**Day Registration Entitlements**

- Entry to all sessions on the day of registration
- Lunch, morning and afternoon tea on the day of registration
- Attendance at evening functions on the day of your registration (dinners & poster sessions)
- Access to the electronic abstracts
- Breakfast for the day of your attendance

## CONFERENCE APP

The 2019 BacPath Mobile App will keep you organised during the meeting, allowing you to (i) view the full conference program; (ii) view all abstracts for the conference; and (iii) save your favourite sessions and plan your day.

To get the 'App', please open the following link in your internet browser on your phone, iPad or laptop. http://bacpath-2019.m.asnevents.com.au/. You can then save the page to your home screen; the conference logo will then appear as an icon on your home screen for you to open as an App. You must 'log in' each day to utilise all of the functions within the Conference App. Simply enter the same email & password which you used to register.

## WI-FI ACCESS

Complimentary conference wifi is available, no password required.

# DELEGATE INFORMATION

## SPEAKER PREPARATION INSTRUCTIONS

Presenters are requested to bring their presentation on a USB with them to the conference and upload it on the presentation laptop in the lecture room before the session starts. Please allow at least a full session before yours to upload your presentation. The room comes with a lectern, a PC equipped with Microsoft Office, data projection (**16:9 aspect ratio**), microphone and audio and a pointer. Internet access will be available in the lecture room.

## POSTER SESSIONS

You should have received notification of your poster session via email. If you are unsure of your poster session, please see the poster listing in the back of the book. Your abstract number is available in the poster listing and also on the Conference App. It is advised that all poster presenters stand by their poster during their allocated session. **Posters should only be displayed on the day of your poster session.** Please make sure your poster is displayed on the morning of your allocated poster session and then taken down at the end of the day. Velcro is available on the poster boards and also at the registration desk to fix your poster to the boards.

**Poster Session I**

- **Date:** Monday 30th September 2019
- **Time:** 8:00 PM - 10:00 PM
- **Location:** Samuel Copley Room

**Poster Session II**

- **Date:** Tuesday 1st October 2019
- **Time:** 8:00 PM - 10:00 PM
- **Location:** Samuel Copley Room

## SOCIAL MEDIA

During BacPath's biannual molecular bacteriology conference, many of our speakers will be presenting exciting novel research that is not yet published. While the conference has an active social media presence, we respect the speakers' right to request that their work not be shared across social media. The sharing of data without the speakers' consent on publicly accessible platforms may prevent its subsequent publication in scientific journals and compromise their scientific progress. In light of this, BacPath's social media policy during meetings is as follows:

- Following BacPath on Twitter: **@BacPath**, and tweeting about the meeting using any relevant conference hashtags such as **#BacPath15**

All talks are "tweetable" by default, but speakers can explicitly request that certain talks, slides, or findings be left out of the social media conversation. The session chairs will provide clear instructions at the beginning of each talk to highlight any such speaker requests.

We ask all attendees to refrain from:

- Recording or reproducing audio, video, or photos of any content presented at oral or poster sessions within the BacPath meeting.
- Collecting or distributing this content without the presenter's permission is strictly prohibited.
- The use of rude and profane language to engage in slander or personal attacks across social media platforms

# SOCIAL PROGRAM

### Poster Session I/Drinks

- **Date:** Monday 30th September 2019
- **Time:** 8:00 PM - 10:00 PM
- **Location:** Samuel Copley Room
- **Cost:** Included in the registration fee

### BBQ Dinner

- **Date:** Tuesday, 1st October 2019
- **Time:** 6:30 PM - 8:00 PM
- **Location:** Garden Pavilion
- **Cost:** Included in the registration fee

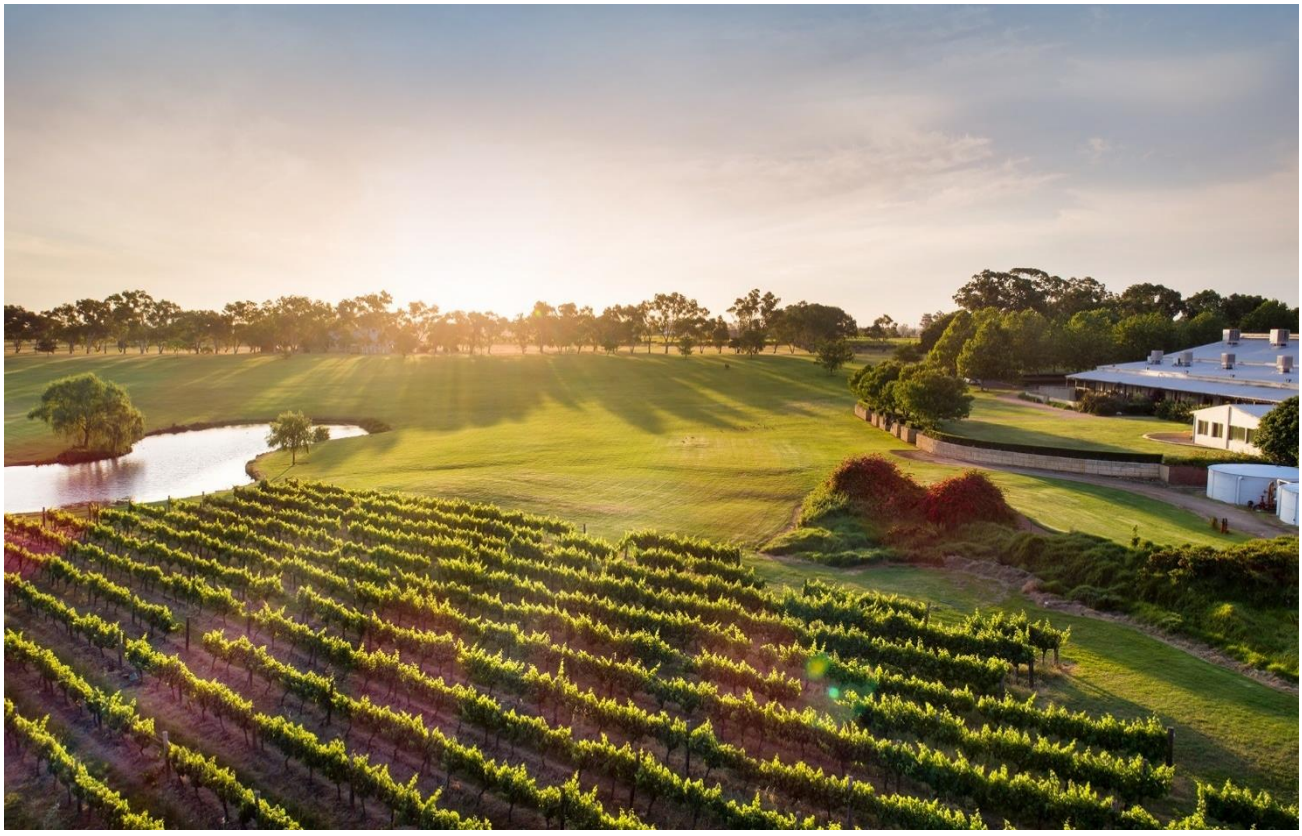
### Poster Session II/Drinks

- **Date:** Tuesday 1st October 2019
- **Time:** 8:00 PM - 10:00 PM
- **Location:** Samuel Copley Room
- **Cost:** Included in the registration fee

### Conference Dinner

- **Date:** Wednesday 2nd October 2017
- **Time:** 5:30 PM - 10:00 PM
- **Location:** Sandalford Wines
- **Cost:** Included in the registration fee

  *Enquire about additional tickets at the registration desk*



# SOCIAL PROGRAM

### Offsite Tours

The BacPath15 committee is delighted to be able to provide complimentary offsite tours to all our delegates during the free time session on Wednesday afternoon. The Swan Valley is abundant in award-winning wineries, breweries and providore vendors which we hope to showcase to our delegates with these offsite tours. Three tour alternatives are on offer; a beer, wine or providore.

## BEER

*Beer paddle tastings available*

The beer tour departs the Vines Resort at 2:00 PM on Wednesday and takes delegates to MASH Brewing first. MASH brewing began in 2006 and since has been named Best WA Brewery 2 years in a row and as well as winning numerous AIBA awards across a large range of categories including a gold medal for their XPA and their IPA Copy Cat. Delegates will be able to taste a range of beers provided by MASH brewing on tasting paddles.

After tasting our way through the beers that MASH has to offer, delegates will move their way down the road to Duckstein Brewery, which pair's German-style food with equally delicious beers. Established in 2000, Duckstein was one of the pioneer microbreweries in the Swan Valley and their beer is made in the ancient tradition of Reinheitsgebot, meaning only water, malt and hops are used to produce beer that is both additive and chemical-free. Delegates will be able to enjoy a tasting paddle of Duckstein's finest beers.


## FOOD

*Chocolate, Honey and Mead tastings*

The providore tour departs the Vines Resort at 2:00 PM on Wednesday and takes delegates to the Margaret River Chocolate Co. The chocolate factory houses all things chocolate with tastings available to our delegates. Opening in 1999, Margaret River Chocolate Co has won numerous awards for their chocolate and we encourage delegates to make the most of the free tastings. The sister company Margaret River Providore is located on-site and delegates can make the most of their tastings of preserves, chutneys, olive oils, wines and even chocolate liqueurs. Following the chocolate sampling, delegates will make their way to the House of Honey where there will be samplings of locally produced honey. Did you know that two pots of honey can taste completely different depending on which species of nectar the bees visit? In addition to honey there will also be mead tastings available for those which wish to participate.

THE MARGARET RIVER CHOCOLATE CO

THE HOUSE OF HONEY

Bees in the Valley

## WINE

*Wine tastings at both locations*

The wine tasting tour will depart from the Vines Resort just before 2:00 PM and make its way to Upper Reach Winery. Wine tastings will take place on the Lawn Terrance in a relaxed atmosphere where delegates will be able to have some fun and play a few games while tasting the wine on offer at Upper Reach Winery, or just sit in the sun while enjoying great wine. Following the tastings at Upper Reach Winery delegates will move to Sittella Winery which was established in 1998. It has won awards for its sparkling, white and red wines. Overlooking stunning vineyards delegates will be able to taste their way through the Sittella range of wines.

UPPER REACH

handcrafted wines · swan valley

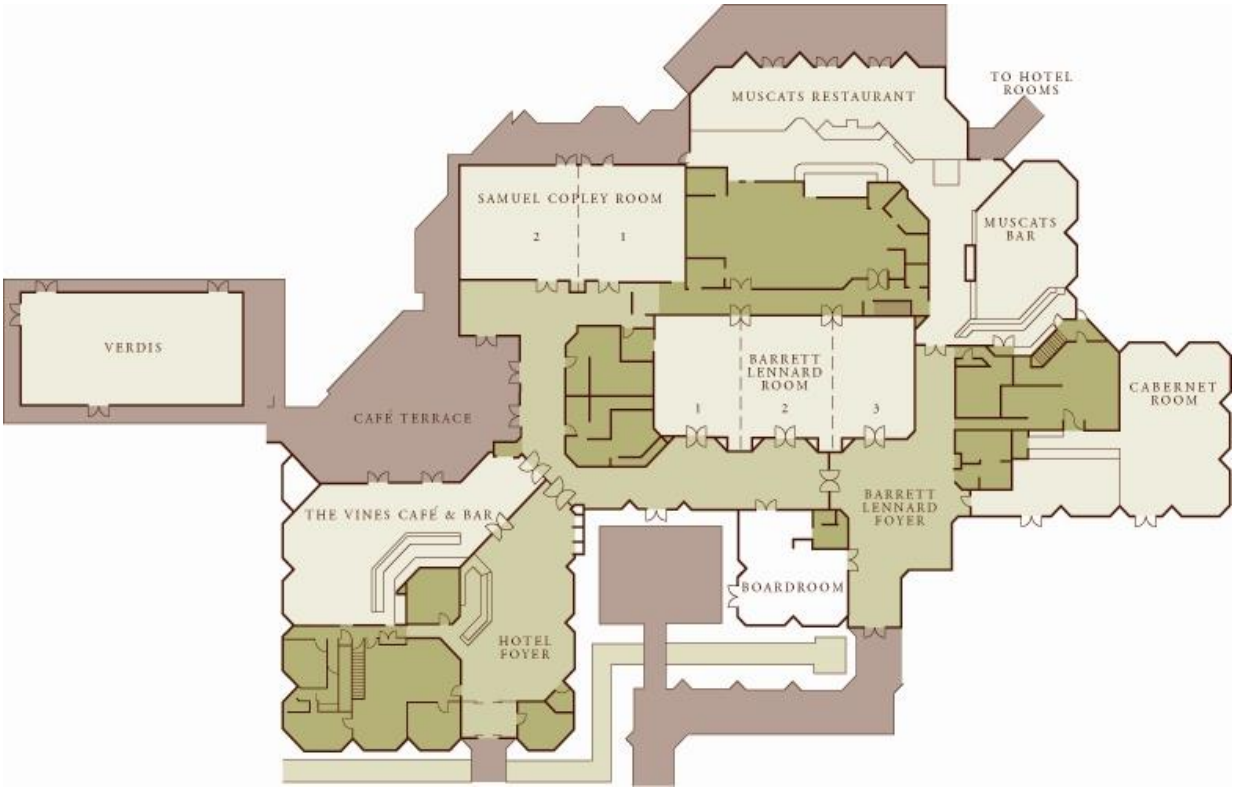
ESTD 1998

SITTELLA

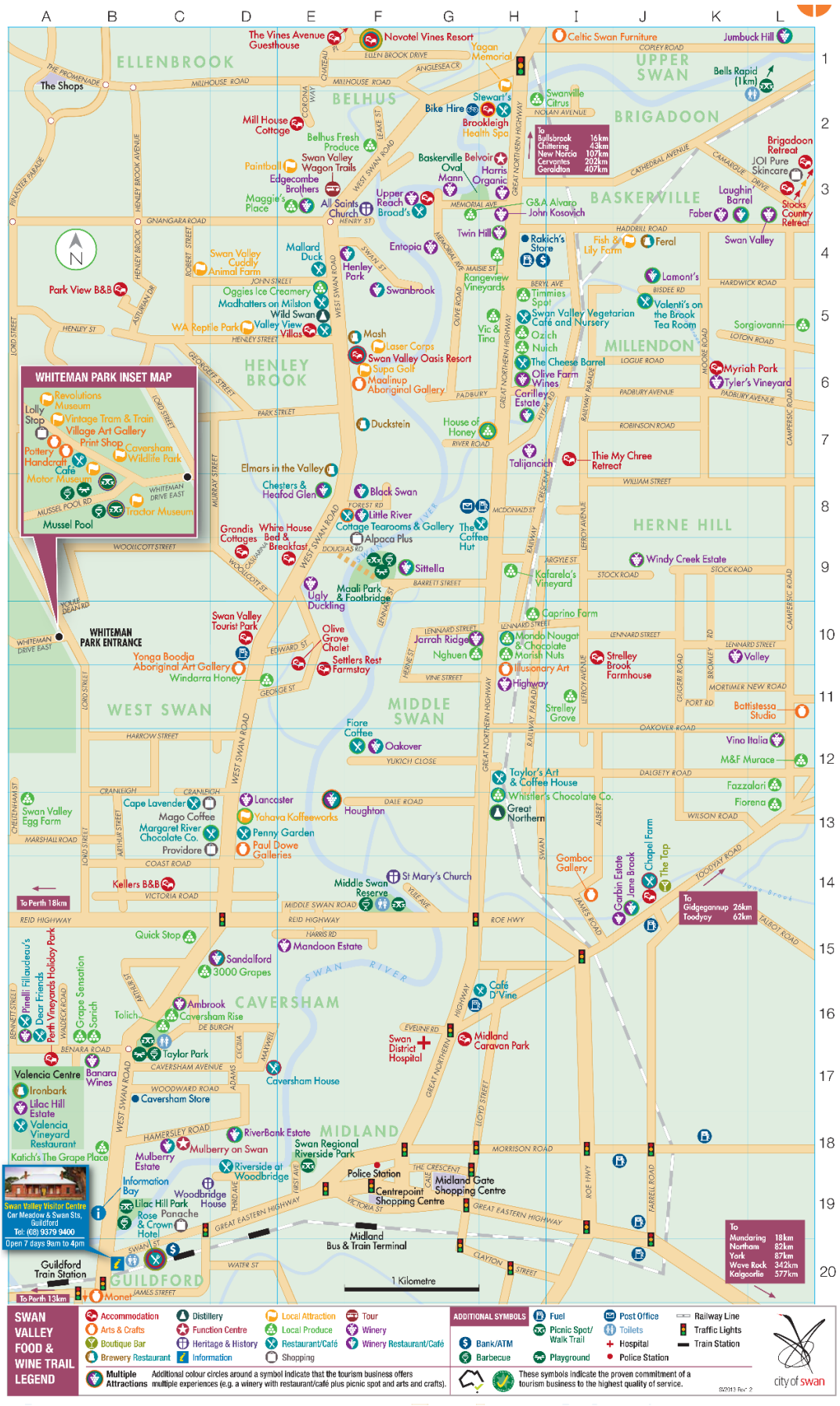
# THE NOVOTEL VINES RESORT AND COUNTRY CLUB

www.vines.com.au/The-Resort

# THE NOVOTEL VINES RESORT AND COUNTRY CLUB

# SWAN VALLEY FOOD AND WINE TRAIL MAP



# SCIENTIFIC PROGRAM

## Monday 30th September 2019

*Barrett Lennard Room*

### Opening Address

5:30 PM – 5.40 PM

### Keynote Speaker 1

5:40 PM - 6:25 PM

*Introduction: Charlene Kahler*

5:40 PM **Ann Jerse**
Tit for tat: Antibiotic resistance, microbial fitness and back again *abs# 1*

### Session 1: Host-pathogen Interactions I

6:25 PM – 7:45 PM

*Chairs: Hayley Newton and Tim Barnett*

*Session sponsor: Institute for Glycomics, Griffith University*

6:25 PM **Timothy Wells**
Mechanisms governing the antibody-mediated exacerbation of *Pseudomonas aeruginosa* infection. *abs# 2*

6:45 PM **Stephan Brouwer**
Scarlet fever-causing *Streptococcus pyogenes* require streptolysin O, host cytosolic glutathione and prophage exotoxins for nasopharyngeal infection *abs# 3*

7:00 PM **Jonathan G Williams**
*Streptococcus pyogenes* causes polymorphonuclear leukocyte dysfunction, contributing to inflammation *abs# 4*

7:15 PM **Emily Kibble**
Macrophage Infectivity Potentiator-like Proteins Affect Virulence of *Neisseria meningitidis abs# 5*

7:30 PM **Yi Wei Lee**
Exploring the functional interactions between *Coxiella burnetii* Dot/Icm effectors *abs# 6*

### Rapid Posters I

7:45 PM - 8:00 PM

*Chairs: Hayley Newton and Tim Barnett*

7:45 PM **Janine Hofmann**
Characterising novel metabolic pathways in *Coxiella burnetii*, the causative agent of the zoonotic disease Q fever *abs# 7*

7:48 PM **Amy Pham**
High prevalence of antibodies that inhibit killing of *Pseudomonas aeruginosa* in a cystic fibrosis cohort *abs# 8*

7:51 PM **Robson Kriiger Loterio**
Intracellular modulation by *Coxiella burnetii* effectors in macrophages *abs# 9*

7:54 PM **Joanna E Musik**
Utilising a poison pill for the study of secretion systems *abs# 10*

7:57 PM **Vassiliy N. Bavro**
Structural and functional characterisation of the gating mechanism of the outer membrane factor MtrE from *Neisseria gonorrhoeae abs# 11*

## Poster Session I/Drinks

8:00 PM - 10:00 PM
*See page 30 for details*

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# Tuesday 1st October 2019

*Barrett Lennard Room*

## Perth Convention Bureau Speaker

8:30 AM - 9:15 AM
*Introduction: Geoff Coombs*

8:30 AM **Paal Andersen**
The nasal microbiome and Staphylococci *abs# 12*

## Session 2: Omics

9:15 AM – 10:35 AM
*Chairs: Nichollas Scott and Stephanie Neville*

9:15 AM **Erin P. Price**
Unveiling Microbial Diversity and Activity in Cystic Fibrosis Sputa Using Microbial Metatranscriptomics *abs# 13*

9:35 AM **Swaine L Chen**
Genomic insights into a totally new disease caused by *Streptococcus agalactiae* in Southeast Asia *abs# 14*

9:50 AM **Jake A Lacey**
Rapid transmission and replacement of diverse *Streptococcus pyogenes* isolates along household and community networks in rural Indigenous communities *abs# 15*

10:05 AM **Samantha J Munn**
Transcriptomic, proteomic and functional analysis of the plasmid pCS1-1 during spore formation in *Clostridium sordellii abs# 16*

10:20 AM **Brandon M Sy**
Revealing the pathogen-specific RNA-binding proteome using UV-crosslinking *abs# 17*

## Morning Tea
10:35 AM - 11:05 AM

## Session 3: Microbes and Metals
11:05 AM - 12:25 PM
*Chairs: Timothy Wells and Amy Cain*

11:05 AM **Stephanie L Neville**
The role of glutathione in *Streptococcus pneumoniae* copper resistance at the host-pathogen interface. *abs# 18*

11:25 AM **Matthew Sullivan**
Copper and zinc resistance mechanisms contribute to Group B streptococcal virulence and pathogenesis *abs# 19*

11:40 AM **Elena Colombi**
Role of Integrative Conjugative Elements in the evolution of the kiwifruit pathogen *Pseudomonas syringae* pv. *actinidiae abs# 20*

11:55 AM **Cheryl-Lynn Y. Ong**
The role of zinc acquisition and zinc tolerance in Group A Streptococcal infection *abs# 21*

12:10 PM **Saleh F. Alquethamy**
*Acinetobacter baumannii* employs multiple pathways for zinc and cadmium efflux *abs# 22*

## Lunch
12:25 PM - 1:35 PM

## Visiting Raine Professor
1:35 PM - 2:20 PM
*Introduction: Mitali Sarkar-Tyson*

1:35 PM **Tim Atkins**
Marmoset model of melioidosis *abs# 23*

## Session 4: Host-pathogen Interactions II
2:20PM - 3:40PM
*Chairs: Aimee Tan and Jai Tree*
*Session sponsor: Institute for Glycomics, Griffith University*

2:20 PM **Nichollas E Scott**
Protein *O*-glycosylation is conserved in *Burkholderia* species and results in the generation of *O*-glycosylation glycan specific antibodies during human infections *abs# 24*

2:40 PM **Steven Mileto**
Acute *Clostridioides difficile* gastrointestinal infection induces systemic disease symptoms, in a toxin and time-dependent manner *abs# 25*

2:55 PM **Amy Bottomley**
Investigating the role of a novel cell division protein, YtfB, in eukaryotic cell adhesion *abs# 26*

3:10 PM **Bhavna Padmanabhan**
Formation of the spacious *Coxiella*-containing vacuole depends on host transcription factors TFEB and TFE3. *abs# 27*

3:25 PM **Muhammad Kamruzzaman**
Spreading antibiotic tolerance in the Enterobacteriaceae: a novel toxin-antitoxin system in major resistance plasmids confers antibiotic and heat tolerance and promotes biofilm formation. *abs# 28*

## Afternoon Tea
3:40 PM - 4:10 PM

## Session 5: Bacteriophage
4:10 PM - 5:15 PM
*Chairs: Elena Colombi and Carola Venturini*

4:10 PM **Trevor Lithgow**
A pipeline for bacteriophage (phage) discovery, characterization and pre-clinical evaluation. *abs# 29*

4:30 PM **Carola Venturini**
Takes two to tango: characterizing the interactions between *Klebsiella pneumoniae* and its phages. *abs# 30*

4:45 PM **Bethany Bowring**
Characterization of an equine *Klebsiella pneumoniae* infection using *de novo* isolated bacteriophages *abs# 31*

5:00 PM **Barakat Alsuwayyid**
Genetic diversity and distribution of filamentous prophage in *Neisseria abs# 32*

## Rapid Posters II
5:15 PM - 5:30 PM
*Chairs: Elena Colombi and Carola Venturini*

5:15 PM **Edward Mikucki**
Serogroup W clonal complex 11 meningococci from Western Australia have increased invasive potential compared to other hyperinvasive lineages. *abs# 33*

5:18 PM **Aimee Tan**
Zinc homeostasis in *Klebsiella pneumoniae abs# 34*

5:21 PM **Marina L Zupan**
Elucidating the Zn(II)-binding mechanism of *Streptococcus pneumoniae* AdcAII *abs# 35*

5:24 PM **Clare L Moran**
Trehalase Activity Supports *Burkholderia pseudomallei* Virulence and External Survival *abs# 36*

5:27 PM **Liam K. R. Sharkey**
Defining the regulon of WalKR; the only essential two-component system in *Staphylococcus aureus abs# 37*

## Free Time
5:30 PM - 6:30 PM

## Tuesday BBQ Function
6:30 PM - 8:00 PM

## Poster Session II/Drinks
8:00 PM - 10:00 PM
*See page 32 for details*

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# Wednesday 2nd October 2019
*Barrett Lennard Room*

## Keynote Speaker 2
8:30 AM - 9:15 AM
*Introduction: Dan Knight*

8:30 AM **Richard W Titball**
Epsilon toxin, Mal and multiple sclerosis *abs# 38*

## Session 6: Molecular Pathogenesis I
9:15 AM - 10:35 AM
*Chairs: John Atack and Martina Sanderson-Smith*
*Session sponsor: Curtin Health Innovation Research Institute*

9:15 AM **Alistair Standish**
Tyrosine phosphorylation modulates OmpR and H-NS virulence regulation in *Shigella flexneri* and *Escherichia coli abs# 39*

9:35 AM **Aleksandra W Debowski**
The macrophage infectivity potentiator protein: Evaluation of a novel target in Gram-negative bacteria for therapeutic intervention. *abs# 40*

9:50 AM **Zachary N Phillips**
Phase-variable regulation in *Streptococcus pneumoniae* pathobiology and vaccine development *abs# 41*

10:05 AM **Michael A Burch**
The role of regulatory RNAs in *Acinetobacter baumannii abs# 42*

10:20 AM **Daniel G Mediati**
Regulatory RNA interactome of methicillin-resistant *Staphylococcus aureus* reveals genes required for antibiotic tolerance. *abs# 43*

## Morning Tea
10:35 AM - 11:05 AM

## Session 7: Antimicrobial Resistance I
11:05 AM - 12:25 PM
*Chairs: Erin Price and Dan Knight*
*Session sponsor: The University of Sydney*

11:05 AM **Francisco Garcia-Del Portillo**
Salmonella peptidoglycan biosynthesis inside eukaryotic cells: novel enzymes impacting antibiotic resistance *abs# 44*

11:25 AM **Derek S Sarovich**
ARDaP: Antimicrobial Resistance Detection and Prediction from Whole-Genome Sequence Data *abs# 45*

11:40 AM **Rhys T. White**
The Emergence of Pandemic Fluoroquinolone-Resistant Uropathogenic *Escherichia coli* Clones in Australia *abs# 46*

11:55 AM **Minh Duy Phan**
Contribution of plasmid-mediated ciprofloxacin resistance to the fitness of *Escherichia coli* ST131 *abs# 47*

12:10 PM **Karina Yui Eto**
Mobilization of Antimicrobial-Resistance Plasmids in *Staphylococcus aureus abs# 48*

## Lunch
12:25 PM - 1:45 PM

## Free Time/Organised Tours
1:45 PM - 4:00 PM

## Buses to Sandalford Wines

Depart 5:30 PM

## Pre-dinner Drinks

6:00 PM - 6:40 PM

## BacPath/ASM WA Chair Address

6:40 PM - 6:50 PM
*Chair: Charlene Kahler*

## ASM BacPath Oration

6:50 PM - 7:30 PM
*Introduction: Charlene Kahler*

6:50 PM **Mark Schembri**
Understanding uropathogenic *E. coli* resistance and virulence. *abs# 49*

## Conference Dinner

7:30 PM - 10:00 PM

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# Thursday 3rd October 2019

*Barrett Lennard Room*

## Keynote Speaker 3

8:30 AM - 9:15 AM
*Introduction: Joshua Ramsay*

8:30 AM **Susanne Gebhard**
Need-based activation of antibiotic resistance *abs# 50*

## Session 8: Antimicrobial Resistance II

9:15 AM - 10:35 AM
*Chairs: Faye Morris and Derek Sarovich*
*Session sponsor: The University of Sydney*

9:15 AM **Bart A Eijkelkamp**
Antimicrobial fatty acids impact membrane biology and antibiotic resistance in *Acinetobacter baumannii abs# 51*

9:35 AM **Keith A Stubbs**
Overcoming antibiotic resistance: Inhibiting the AmpC β-Lactamase Induction Pathway *abs# 52*

9:50 AM **Anna Roujeinikova**
Sulfonamide inhibitors of carbonic anhydrases impact multiple targets *abs# 53*

10:05 AM **Riley JT Murphy**
A novel mechanism for translational regulation of linezolid resistance in *Staphylococcus aureus abs# 54*

10:20 AM **Sue Chin Nang**
Polymyxin resistance: Deciphering the interplay of different lipid A modifications in *Klebsiella pneumoniae abs# 55*

## Morning Tea
10:35 AM - 11:05 AM

## Session 9: Molecular Pathogenesis II
11:05 AM - 12:25 PM
*Chairs: Swaine Chen and Amy Bottomley*
*Session sponsor: Curtin Health Innovation Research Institute*

11:05 AM **Jai J Tree**
A regulatory small RNA is embedded within the Shiga toxin transcript *abs# 56*

11:25 AM **Yogitha N Srikhanta**
Anti-sporulation strategies targeting *Clostridioides difficile* and other spore-forming bacterial pathogens *abs# 57*

11:40 AM **Nicole M Bzdyl**
Peptidyl-prolyl isomerase, *ppiB*, is essential for proteome homeostasis and virulence in *Burkholderia pseudomallei abs# 58*

11:55 AM **Jinki Yeom**
Protein longevity as a wake-up call for dormant cells *abs# 59*

12:10 PM **Felise G Adams**
Biological insights into the mechanisms that define the lipidomic landscape of *Acinetobacter baumannii abs# 60*

## Closing remarks/Awards/BacPath16 announcement
12:25 PM - 12:40 PM

## Lunch
12:40 PM - 2:00 PM


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# POSTER LISTING

## POSTER SESSION 1

**Monday 30th September, 8:00 PM-10:00 PM, Samuel Copley Room**

**Laura Álvarez Fraga**
Differential role of the K1 and K5 capsule in virulence of the fluoroquinolone-resistant uropathogenic *Escherichia coli* ST1193 clone *abs# 102*

**John M Atack**
Multiple bacterial veterinary pathogens contain phase-variable regulons; phasevarions. *abs# 103*

**Tim C Barnett**
Genetic basis of Group A *Streptococcus* cotrimoxazole resistance *abs# 104*

**Manasa Bharathwaj**
Investigating the Tat translocation of β-lactamases in Gram-negative bacteria *abs# 105*

**Melissa H Brown**
Structure and function of the staphylococcal multidrug efflux pump QacA: the importance of helix 12 *abs# 106*

**Amy K Cain**
Transposon Directed Insertion Sequencing (TraDIS) to investigate genes required for survival during antimicrobial synergy in multidrug-resistant *Klebsiella pneumoniae abs# 107*

**Chiara Carnevali**
Disseminated Tuberculosis (TB); A Potential Role for the Antigen 85 Complex. *abs# 108*

**Jackie K Cheung**
EngCP, an endo α-*N*-acetylgalactosaminidase, is involved in the virulence of *Clostridium perfringens abs# 109*

**Shu Sin Chng**
Metabolic labelling and affinity-based protein profiling reveal putative transporters for the Pseudomonas Quinolone Signal *abs# 110*

**Nural Cokcetin**
Unlocking the secrets of an ancient antimicrobial, honey, using modern transcriptomics *abs# 111*

**Nicholas V Coleman**
A new versatile broad host range cloning and expression vector for Gram-negative bacteria *abs# 112*

**Bliss A Cunningham**
Host mobilisation of zinc in response to pneumococcal infection *abs# 113*

**Danielle D'Souza**
CppA: A putative major fimbrial protein and novel virulence factor for clostridial myonecrosis? *abs# 114*

**Mark R Davies**
Enhancing group A streptococcal vaccine design through global population genomics and non-human primate infection studies. *abs# 115*

**Alicia Fajardo-Lubian**
Bacteriophage isolation against common multidrug resistant human pathogens *abs# 116*

**Brian M Forde**
Routine genomic surveillance to track treat and prevent Healthcare-associated infections *abs# 117*

**Katherine Ganio**
Biochemical and structural characterisation of the *Haemophilus influenzae* PsaA ortholog, HIPsaA *abs# 118*

**Sarah C Geiger**
Understanding clonal expansions from a population genetics perspective *abs# 119*

**Evan Gibbs**
Structural and Functional Analysis of a Representative PACE protein *abs# 120*

**Jessica Gray**
Exploring gene essentiality in *Klebsiella pneumoniae abs# 121*

**Steven J Hancock**
Genetic Basis and Regulatory Control of IncC Plasmid Conjugation *abs# 122*

**Leila Jebeli**
DNA contributes to the stability of biofilms formed by *Klebsiella pneumoniae* AJ094 *abs# 123*

**Ruiting Lan**
Surfaceome analysis of Australian epidemic *Bordetella pertussis* reveals potential vaccine antigens *abs# 124*

**Ruiting Lan**
Genomic epidemiology of erythromycin-resistant *Bordetella pertussis* in China *abs# 125*

**Daniel R Laucirica**
Polymicrobial infection and neutrophilic disease in cystic fibrosis airways *abs# 126*

**Liping Li**
Transposon-Insertion Sequencing reveals the genetics underpinning bacterial tolerance of common hospital and household biocides *abs# 127*

**Eric Mandela**
The Gram-negative bacterial periplasm; Architecture significance *abs# 128*

**Elisa Massella**
*Escherichia coli* from companion animals, livestock, wildlife and food as potential sources of antimicrobial resistance and virulence associated genes *abs# 129*

**Diane McDougald**
*Vibrio cholerae* in food vacuoles expelled by protozoa are protected from stresses and more infectious *in vivo* than free-living cells *abs# 130*

**Karla A Mettrick**
Global transcriptional profiles in RND efflux pump mutants *abs# 131*

**Jamie-Lee Mills**
Modelling natural immunity to *Streptococcus pyogenes* skin and mucosal infections in a mouse model *abs# 132*

# POSTER LISTING

## POSTER SESSION 2

**Tuesday 1st October, 8:00 PM-10:00 PM, Samuel Copley Room**

**Joshua N Monteith**
A novel method of O-antigen-specific antibody purification *abs# 201*

**Faye C Morris**
The role of lactate metabolism in *Acinetobacter baumannii* pathogenesis *abs# 202*

**Varsha Naidu**
Uncovering the mechanisms of resistance in a multi-drug resistant *Acinetobacter baumannii* isolate *abs# 203*

**Renee N Ng**
Exploring the therapeutic potential of phage therapy to treat *Pseudomonas aeruginosa* infection in people with cystic fibrosis *abs# 204*

**Nhu TK Nguyen**
Complex multi-level control of hemolysin production by uropathogenic *Escherichia coli abs# 205*

**Kaori Ohtani**
VirY, a small regulatory RNA that regulates toxin production in *Clostridium perfringens abs# 206*

**Tugce Onur**
Characterization of *Escherichia coli Adhesins abs# 207*

**Thaisy Eliza Pacheco dos Santos**
Does quorum-sensing regulated by iron in *Klebsiella pneumoniae*? *abs# 208*

**Pawan Parajuli**
Study on the role of O-antigen modifying glucosyltransferase (*gtr*) genes in *Shigella flexneri* serotype 1c virulence *abs# 209*

**Sacha Pidot**
Identification and mobilization of the brasiliquinone biosynthesis gene locus from a human-pathogenic isolate of *Nocardia brasiliensis abs# 210*

**Alaska Pokhrel**
The AceI multidrug transporter plays an important role in virulence of the nosocomial pathogen *Acinetobacter baumannii abs# 211*

**Emma-Jayne Proctor**
Characterising the function of the M-related protein in *Streptococcus pyogenes* virulence *abs# 212*

**Maria Graciela Pucciarelli**
Regulatory RNA contributing to *Listeria monocytogenes* cold adaptation *abs# 213*

**Scott A Rice**
Control of *Pseudomonas aeruginosa* infections using a biofilm targeting, nitric oxide releasing prodrug *abs# 214*

**Natalia C Rosas Bastidas**
Molecular evolution of imipenem-resistance in clinical isolates of *Klebsiella* spp *abs# 215*

**Cheryll M Sia**
The Application of Whole Genome Sequencing in the Prediction of Phenotypic Antimicrobial Resistance in Non-typhoidal *Salmonella* Circulating Australia *abs# 216*

**Thomas Smallman**
Using transposon-directed insertion site sequencing to investigate *Pasteurella multocida* pathogenesis *abs# 217*

**KARA STAUNTON**
Unravelling the function of the non-essential components of the BAM complex using Transposon Directed Insertion Site Sequencing. *abs# 218*

**Miljan Stupar**
Phenotypic characterisation of the LirAB two-component system of *Mycobacterium tuberculosis abs# 219*

**Geraldine J Sullivan**
Detailed genomic analysis of *Pseudomonas aeruginosa* piliated reference strain PAK *abs# 220*

**Matthew J Sullivan**
Flagellin from uropathogenic *E. coli* induces IL-10 during acute urinary tract infection *abs# 221*

**Greg Tram**
Characterisation of the binding affinity of a number of outer-membrane proteins of the pathogen *Haemophilus influenzae* biogroup *aegyptius*, the cause of the lethal febrile disease Brazilian Purpuric Fever *abs# 222*

**Sylvania Wu**
High-resolution transcriptomes of a methicillin-resistant *Staphylococcus aureus* clinical strain during antibiotic responses *abs# 224*

**Winton Wu**
Small RNA networks and vancomycin tolerance in *Staphylococcus aureus abs# 225*

**Alex R Carey Hulyer**
How isscarce and essential manganese acquired by *Streptococcus Pneumoniae* at the host-pathogen interface? *abs# 226*

**Yaramah M Zalucki**
Evolution of improved secretion, function and fitness is driving the emergence of new alleles of the NDM-1 drug resistance gene. *abs# 227*

**Hassan M. Al-Emran**
Multidrug resistance analysis of community acquired urinary tract infections in the coastal region of Bangladesh *abs# 101*

**Scott W Mitchell**
Class 1 integrons and antibiotic resistance genes in wild and domestic horses. *abs# 228*

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# PRIZES

## Poster Prizes

**American Society for Microbiology Poster Prize**
Session: Poster Session I
Value: ASM membership for 1 year and a $200 ASM book voucher

**BacPath Poster Prize**
Session: Poster Session II
Value: $300

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Session: Rapid Poster Session II
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## Grand Prize

**FEMS Pathogens and Disease Best Oral Presentation Prize**
Sessions: All oral sessions excluding rapid posters
Value: $500

All prizes will be awarded during the closing session (Thursday 3rd October, 12:25 PM - 12:40 PM)

# ABSTRACTS

**1**

## Tit for tat: Antibiotic resistance, microbial fitness and back again

**Ann Jerse**[1]

*1. Uniformed Services University, TBC, United States*

Dr. Jerse has worked in the area of bacterial pathogenesis for over 30 years and on *Neisseria gonorrhoeae* since 1991 with a focus on gonococcal adaptation to the genital tract using human and murine infection models. Her laboratory is also very active in the development of vaccines, vaginal microbicides and novel anti-infectives, which are needed to combat the serious problem of antibiotic resistance in *N. gonorrhoeae*. To better understand the spread of antibiotic resistance in the gonococcus, Dr. Jerse studies the relationship between antibiotic resistance genes and gonococcal fitness *in vitro* and *in vivo*. In this presentation she will discuss how some antibiotic resistance alleles (i.e. *mtr* mutations, *gyrA* mutations, mosaic *penA* alleles) confer a fitness benefit or cost to the gonococcus and how compensatory evolution can rescue fitness disadvantages that accompany some resistance mutations. This line of investigation helps elucidate the spread of antibiotic resistant strains and may explain why resistant strains can be maintained within communities in the absence of antibiotic pressure.

**2**

## Mechanisms governing the antibody mediated exacerbation of *Pseudomonas aeruginosa* infection.

**Amy Pham[1], Carrie Coggon[1], Joshua Monteith[1], Emma Ledger[1], Ian Henderson[2], Daniel Chambers[3], Daniel Smith[4], Timothy Wells[1]**

*1. University of Queensland Diamantina Institute, Brisbane, QLD, Australia*

*2. Institute of Molecular Bioscience, The University of Queensland, Brisbane, QLD, Australia*

*3. University of Queensland, Brisbane, QLD, Australia*

*4. Adult CF centre, Prince Charles Hospital, Brisbane, QLD, Australia*

*Pseudomonas aeruginosa* is the principle pathogen implicated in progressive and recurrent respiratory infections in cystic fibrosis. Recently, we described impaired serum-mediated killing of *P. aeruginosa* in patients with bronchiectasis that was associated with increased severity of respiratory infections. This inhibition was mediated by high titres of O-antigen-specific IgG2 antibodies. These 'inhibitory antibodies' (iAbs) have been found in patients with bronchiectasis (24%), cystic fibrosis (33%) and post lung transplant (30%). Importantly, three patients with *P. aeruginosa* infection and iAbs have been successfully treated with plasmapheresis. All patients had immediate benefit from this treatment with a significant drop in hospitalisations, antibiotic use and markers of inflammation.

Here we determine the mechanisms that underlie the inhibition phenotype. We demonstrate that inhibitory antibodies can be of other subtypes such as IgA, but must be of high affinity. Inhibition does not seem to be dependent on LPS serotype, with IAbs specific to all prevalent *P. aeruginosa* serotypes having been detected. Purified O-antigen of various serotypes can also induce 'inhibitory antibodies' in a mouse model. Finally, LPS density and length are critical factors in the inhibition phenotype. We have demonstrated that biotinylated O-antigen can be used to specifically remove inhibitory antibodies in patient serum, restoring the bactericidal effect. These results will improve methods to detect and treat inhibitory antibodies. Finding ways to remove or counteract this antibody can lead to improvement in health without the need for antibiotics.

**3**

## Scarlet fever-causing *Streptococcus pyogenes* require streptolysin O, host cytosolic glutathione and prophage exotoxins for nasopharyngeal infection

**Stephan Brouwer[1], Timothy C Barnett[1], Diane Ly[2], Katherine J Kasper[3], David MP De Oliveira[1], Tania Rivera-Hernandez[1], Amanda J Cork[1], Johanna Richter[1], Gordon Dougan[4, 5], Victor Nizet[6], Kwok-Yung Yuen[7], John K McCormick[3, 8], Martina L Sanderson-Smith[2], Mark R Davies[1, 9], Mark J Walker[1]**

*1. Australian Infectious Diseases Research Centre and School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia, QLD, Australia*

*2. Ilawarra Health and Medical Research Institute and School of Biological Sciences, University of Wollongong, Wollongong, NSW, Australia*

*3. Department of Microbiology and Immunology and the Centre for Human Immunology, Schulich School of Medicine & Dentistry, Western University, London, Ontario, Canada*

*4. The Wellcome Trust Sanger Institute, Hinxton, Cambridge, United Kingdom*

*5. University of Cambridge, Cambridge, United Kingdom*

*6. Department of Pediatrics, University of California San Diego, La Jolla, California, USA*

*7. State Key Laboratory for Emerging Infectious Diseases, The University of Hong Kong, Hong Kong Special Administrative Region, Hong Kong , China*

*8. Lawson Health Research Institute, London, Ontario, Canada*

*9. Department of Microbiology and Immunology at the Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Melbourne, Victoria, Australia*

The re-emergence of scarlet fever since 2011 poses a new threat to global public health. A detailed understanding of the processes controlling the upsurge in scarlet fever cases is therefore instrumental in the fight against this disease. The capacity of North Asian serotype M12 (*emm12*) *Streptococcus pyogenes* (Group A *Streptococcus*, GAS) to cause scarlet fever has been linked to the presence of antibiotic resistance elements and novel prophages, including prophage ΦHKU.vir encoding the secreted superantigens SSA, SpeC and the DNase Spd1. However, robust empirical evidence defining the contribution of prophage-encoded exotoxins to the pathogenesis of scarlet fever is lacking. Here we sought to investigate the regulation of these exotoxins and what role they play in GAS nasopharyngeal infection using defined genetic knockouts in a humanized mouse model for pharyngeal infection. A small molecule screen identified biothiols, such as glutathione (GSH), as a factor specifically enhancing secretion and activity of the exotoxin SSA. Our data reveal SSA as the first thiol-activated superantigen to be reported. We show that GSH is released from host cellular stores through the action of the pore-forming toxin streptolysin O, providing a mechanistic framework for how extracellular GAS gains access to highly abundant GSH *in vivo*. This work also provides evidence that DNase Spd1 is required for optimal growth in human blood and confers resistance to neutrophil killing. To examine how these exotoxins contribute to the fitness of GAS, we generated isogenic single and triple knockout mutants in the *ssa*, *speC* and *spd1* genes. Loss of all three exotoxins significantly reduced nasopharyngeal colonization in a mouse infection model, whereas single mutants did not, suggesting a synergistic relationship between these exotoxins. Taken together, these findings offer a new paradigm for GAS pathogenesis and support the hypothesis that acquisition of ΦHKU.vir-encoded exotoxins has played a key role in the selection and expansion of scarlet fever lineages in North Asia.

**4**

## *Streptococcus pyogenes* causes polymorphonuclear leukocyte dysfunction, contributing to inflammation

**Jonathan G Williams[2, 1], Nicholas J Geraghty[2, 1], James A Tsatsaronis[2, 1], Jody Gorman[2, 1], Ronald Sluyter[2, 1], Martina L Sanderson-Smith[2, 1]**

*1. School of Chemistry and Molecular Bioscience, University of Wollongong, Wollongong, NSW, Australia*

*2. Illawarra Health and Medical Research Institute, Wollongong, NSW, Australia*

Invasive infections due to *Streptococcus pyogenes* (Group A *Streptococcus*, GAS) cause tissue degradation and sepsis as a result of dysregulated host immune reactions. Polymorphonuclear leukocytes (PMNs) are the primary responding innate immune cells to infection. PMN dysfunction due to changes in activation, regulated cell-death pathway and clearance is associated with invasive disease. GAS responsible for invasive disease frequently acquire mutations in the two-component control of virulence regulator system sensor protein (covS), increasing GAS resistance to PMN killing and promoting bacterial dissemination. We have undertaken an investigation of the PMN response to covS mutant GAS. We show, for the first time, that GAS promotes activation of caspase-1 in PMNs, suggesting inflammasome activation in response to both wildtype and covS mutant GAS. In addition, PMNs exposed to covS mutant GAS show evidence of delayed cell-death, decreased caspase-3 activation, and retention of CD16 and CD31 expression, compared to PMNs infected with wildtype GAS. These data support a hypothesis that reduced phagocytosis of covS mutant GAS may contribute to delayed PMN clearance during infection. Furthermore, LEGENDplex cytometric bead assay analysis reveals PMNs infected with covS GAS release increased IL-1β but reduced TNF-α, which could further contribute to inflammation. We hypothesise that infection of PMNs with covS mutant GAS results in decreased PMN function, delayed PMN cell death, and a reduction in PMN apoptosis, thereby promoting an inflammatory phenotype and allowing bacterial proliferation. Ineffective PMN function during the early stages of invasive GAS infection may be a contributing factor to the development of sepsis.

**5**

## Macrophage Infectivity Potentiator-like Proteins Affect Virulence of *Neisseria meningitidis*

**Emily Kibble[1, 2], Geoffrey Coombs[2], Charlene Kahler[1], Mitali Sarkar-Tyson[1]**

*1. The Marshall Centre for Infectious Diseases Research and Training, School of Biomedical Science, University of Western Australia, Perth, WA*

*2. Antimicrobial Resistance and Infectious Diseases Research Laboratory, Murdoch University, Perth, WA*

*Neisseria meningitidis* is the bacterial causative agent of invasive meningococcal disease (IMD). The rate of IMD in Australia is increasing, with 2017 showing the highest infection rates since 2007. Macrophage infectivity potentiator (Mip) proteins exhibit peptidyl-prolyl *cis/trans* isomerase activity and are found in a wide range of pathogens. *N. meningitidis* encodes for two putative Mip-like proteins, which is uncommon in comparison to other pathogens which encode for a single Mip protein. Previous work has shown presence of one Mip protein to be important for survival of *N. meningitidis* in whole human blood. It is hypothesised both Mip-like proteins encoded by *N. meningitidis* are important novel anti-virulence targets. Three insertional deletion mutants have been created in the *N. meningitidis* strain NMB; two single mutants, each lacking one of the two putative Mip proteins (NMBΔ*mip1* and NMBΔ*mip2*), and one double mutant (NMBΔ*mip1*Δ*mip2*), lacking both putative Mip proteins. All three mutant strains have been assessed for growth at sub-optimal temperatures and survival within a range of host cells. Deletion of the putative Mip proteins has resulted in decreased survival of *N. meningitidis* at high temperatures. Adhesion of mutant strains to host epithelial cells is also impaired, with attachment rates of NMBΔ*mip1*, NMBΔ*mip2* and NMBΔ*mip1*Δ*mip2* decreased by 50%, 30% and 53% respectively, when compared to the control strain NMB. Reduced survival of mutant strains is observed in macrophages, with survival of NMBΔ*mip1*, NMBΔ*mip2* and NMBΔ*mip1*Δ*mip2* decreased by 69%, 79% and 91% respectively. Both recombinant Mip1 and Mip2 proteins are correctly folded and enzymatically active. This indicates both Mip-like proteins are important in *N. meningitidis* to resist macrophage killing, epithelial cell attachment and survival, as well as growth at sub-optimal temperatures. Mip-like proteins represent important anti-virulence targets in *N. meningitidis.*

**6**

## Exploring the functional interactions between *Coxiella burnetii* Dot/Icm effectors

**Yi Wei Lee[1], Malene L. Urbanus[2], Chen Ai Khoo[1], Patrice Newton[1], Miku Kuba[1], Benedict Pheh[1], Nicole Lau[1], Alexander W. Ensminger[2, 3, 4], Hayley J. Newton[1]**

*1. Department of Microbiology and Immunology, The University of Melbourne at the Peter Doherty Institute for Infection and Immunity, Melbourne, VIC, Australia*

*2. Department of Biochemistry, University of Toronto, Toronto, ON, Canada*

*3. Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada*

*4. Public Health Ontario, Toronto, ON, Canada*

The causative agent of the infection Q fever, *Coxiella burnetii*, is a Gram-negative intracellular bacterial pathogen. In humans, alveolar macrophages constitute a niche for *C. burnetii* intracellular replication, which occurs in a lysosome-derived vacuole termed the *Coxiella*-containing vacuole (CCV). *C. burnetii* harbours a Dot/Icm type IV secretion system that is essential for bacterial intracellular replication. This secretion system delivers an arsenal of approximately 150 effector proteins directly from the bacteria into the human host cytosol. Studies over the past decade have offered much insights into bacterial effector functions, primarily with a focus on effectors manipulating specific host proteins. Recently it has become apparent that bacterial effectors can regulate each other once inside the host cell and this may be the primary role of a subset of effectors. A system-wide screen was performed to identify functional relationships between *C. burnetii* effectors. The nuclear *C. burnetii* effector A (NceA) was found to confer toxicity in *Saccharomyces cerevisiae*, and this toxicity was alleviated by co-expression of two other effectors, named Suppress toxicity of NceA A and B (StnA and StnB). This suggests that StnA and StnB can antagonise the activity of NceA. A yeast two-hybrid screen with a HeLa cell cDNA library identified two putative host binding partners of NceA; tyrosyl-DNA phosphodiesterase 2 (TDP2), a DNA repair enzyme, and stromelysin-1 PDGF-responsive element binding protein (SPBP), a transcription cofactor of various autophagy-related proteins. HeLa cells engineered to express NceA demonstrate reduced levels of the autophagy receptor SQSTM1. We hypothesise that NceA alters host transcription, through interactions with TDP2 and SPBP, impacting host cell autophagy. The antagonistic relationship of StnA and StnB with NceA suggests their potential involvement in the host biological cascades targeted by NceA. Interestingly, a *stnA C. burnetii* mutant displayed a multi-CCV phenotype which also indicates a role for this effector in modulating autophagy. This research aims to progress our understanding of these effectors and their interplay with host autophagy function.

**7**

## Characterising novel metabolic pathways in *Coxiella burnetii*, the causative agent of the zoonotic disease Q fever

**Janine Hofmann[1], Mebratu A. Bitew[1], Miku Kuba[2], Hayley J. Newton[2], Fiona M. Sansom[1]**

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The zoonotic pathogen *Coxiella burnetii* poses a serious threat to global public health. *C. burnetii* replicates intracellularly within a unique vacuole derived from the phagolysosome, known as the *Coxiella*-containing vacuole (CCV). To cause disease, *C. burnetii* must not only survive the bactericidal environment within this vacuole, but also obtain energy and nutrients to replicate. Investigating the metabolic pathways required by *C. burnetii* to survive inside host cells may identify novel therapeutic targets.

Recent stable isotope labelling studies revealed that *C. burnetii* is capable of synthesising lactate, despite the apparent absence of a genetic pathway for lactate production. In this study we are investigating two potential lactate-producing pathways. Malolactic enzymes, found in lactic acid bacteria, convert malate to lactate. Our bioinformatic analysis revealed that CBU0823, currently annotated as a NAD-dependent malic enzyme, possesses 43% identity with the malolactic enzyme of the lactic acid bacterium *Oenococcus oeni*. As *C. burnetii* already possesses a putative malate dehydrogenase, MDH (CBU1241), CBU0823 may function as a malolactic enzyme. Alternatively, CBU1241 may perform a dual function and possess MDH and LDH activity.

We have successfully expressed and purified recombinant CBU0823 and CBU1241 as 6xHis N-terminal fusion and GST N-terminal fusion proteins respectively. *In vitro* enzyme assays demonstrated that CBU1241 possesses MDH activity but lacks LDH activity, at least *in vitro,* and that CBU0823 has malic enzyme activity. Current work is examining the malolactic enzyme activity of CBU0823. Future work will examine the role of this pathway in *C. burnetii* intracellular replication.

**8**

## High prevalence of antibodies that inhibit killing of *Pseudomonas aeruginosa* in a cystic fibrosis cohort

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*Pseudomonas aeruginosa* is the principle pathogen implicated in progressive and recurrent respiratory infections in cystic fibrosis. Recently, impaired serum-mediated killing of *P. aeruginosa* was associated with increased severity of respiratory infections in patients with bronchiectasis. This inhibition was mediated by high titres of O-antigen-specific IgG2 antibodies. These 'inhibitory antibodies' were present in 20% of patients chronically infected with *P. aeruginosa*. Patients with these antibodies have been successfully treated with plasmapheresis. Here, we investigated the prevalence and mechanisms behind 'inhibitory antibodies' in patients with CF and *P. aeruginosa* infection without cognate isolates. IgG2 titres were measured in 75 serum samples obtained from patients with CF against eight *P. aeruginosa* serotypes. To confirm the inhibitory capacity of

serum, serum bactericidal assays were performed. We found that 24 of 75 patients had serum that inhibited healthy control killing of *P. aeruginosa*. Interestingly, in a small number of patients with low IgG2 titres, increased antibody affinity appeared to mediate inhibition of killing. Moreover, in two patients with low IgG2 titres but inhibitory serum, we found that high titres of O-antigen specific IgA also inhibited killing of *P. aeruginosa*. Thus, inhibitory antibodies are highly prevalent (33%) in patients with CF and *P. aeruginosa*. Both titre and affinity of these antibodies is important for the inhibition. IgA specific for O-antigen can also inhibit serum-mediated killing even in the absence of IgG2. Thus, diagnostic screens for 'inhibitory antibodies' will need to account for titre, affinity and multiple isotypes binding to *P. aeruginosa* O-antigen.

**9**

## Intracellular modulation by *Coxiella burnetii* effectors in macrophages

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*Coxiella burnetii* is a Gram-negative bacterium and causative agent of Q fever in humans. The bacterium is highly adapted to infect alveolar macrophages and subvert their functions, including the avoidance of TLR recognition, the inhibition of apoptosis and the modulation of diverse vesicle traffic pathways. Its virulence is dependent on the translocation of bacterial proteins (called effectors) into the host cytoplasm through the Dot/Icm T4BSS, creating a spacious intracellular vacuole that supports bacterial replication. Similar to *Coxiella*, *Legionella pneumophila* virulence depends on its T4BSS, however, *Legionella* is more suitable for genetic manipulation, a feature that supports the use of *Legionella* as surrogate host to express *Coxiella* effectors. We generated a library of 66 *L. pneumophilla flaA* mutants expressing *Coxiella* effectors. We screened this library and identified 3 *Coxiella* effectors that may be involved in the manipulation of macrophage functions. Expression of these 3 effectors by *L. pneumophila* led to decreased pyroptosis (measured by LDH release and pore formation assays) and increased cytokine production (IL-1β and IL-6). Thus, we aim to further study these effectors expressing them in eukaryotic cells, identify the possible eukaryotic partner and obtain *Coxiella* mutants for each effector. This study may help to elucidate the function of these effectors, providing information for our understanding of the evasive mechanisms used by intracellular pathogens to subvert the host cell mechanisms. In addition, this study will lead to the identification of target molecules and pathways to the development of immunological therapies.

**10**

## Utilising a poison pill for the study of secretion systems

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Bacterial proteins destined for export from the cytoplasm by the Sec pathway contain an N-terminal signal peptide. This signal peptide is cleaved by signal peptidase upon translocation. Residues in the signal peptide and mature region adjacent to the signal peptidase cleavage site are important for secretion efficiency. Recently we showed that the presence of aromatic amino acids at the second position after the signal peptidase cleavage site (P2') in *Escherichia coli* lead to accumulation of precursor protein. This precursor protein still contains the signal peptide, indicating inefficient processing by signal peptidase, LepB. Here we further investigate this bias, focusing on a protein found in Bacillus, TasA, which contains a phenylalanine at P2'. TasA is usually cleaved by a non-essential signal peptidase, SipW, which contains a serine-histidine active site. TasA and SipW are present on the same operon and are expressed during sporulation and biofilm formation. The fusion of the TasA signal peptide, including the early mature region, to maltose binding protein is toxic when overexpressed. Stepwise mutations back to maltose binding protein from TasA enables us to understand which residues in the TasA signal peptide causes this toxic effect when expressed in *E. coli*. In addition, a similar fusion protein of TasA to beta-lactamase up to P2' was created. This protein showed a decrease in MIC against ampicillin, as well as only precursor protein visualised by western blot. To study which amino acids are permitted, mutational PCR at each position was done before being transformed and grown on plates containing ampicillin at four times the MIC of TasBla P2'. This analysis showed which amino acids allowed for growth, and to what extent LepB processing had been restored. These studies have allowed us to deepen the understanding on which amino acids at certain positions surrounding the LepB cleavage site are important for processing.

**11**

## Structural and functional characterisation of the gating mechanism of the outer membrane factor MtrE from *Neisseria gonorrhoeae*

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Multiple transferable resistance (mtr) operon in *Neisseria gonhorroeae* encodes the RND efflux-system MtrCDE. This tripartite pump is formed by the outer membrane factor (OMF) MtrE, the periplasmic adapter protein (PAP) MtrC, and the inner-membrane protein (IMP) MtrD.

MtrE shares common protomer organisation with other OMF (TolC-family) members (1). It forms a trimeric channel with a 12-stranded β-barrel embedded in the outer membrane, followed by an unique α-barrel domain, which extends into the periplasmic space. At the periplasmic side, each MtrE protomer has two pairs of helical-hairpins (H3/H4 and H7/H8), which contribute to the formation coiled-coil α-barrel and seal the periplasmic

end of the channel in the resting state. Within the context of the MtrE trimer, the repetition of these helical pairs results in the formation of three pairs of “inter-protomer” and “intra-protomer groves” which are speculated to form the contact surface for the PAP MtrC, interaction with which is supposed to cause both tripartite complex association and dilation of the MtrE aperture.

Despite recent advances in structural biology of the tripartite complexes (2), the exact mechanism of the PAP engagement and channel opening of the MtrE remains unclear. This is further complicated by the significant divergence of MtrE from the canonical OMF TolC, the gating of which is relatively well-studied.

Here, we have employed an integrative structural biology approach for the identification of the critical gating residues supporting the closed and open state of the MtrE FA19. By combining site-directed mutagenesis and phenotypic characterization of efflux mutants alongside X-ray crystallography and modelling approaches we provide novel structural model of its gating, which appears markedly different from the rest of the TolC family (3). Furthermore, the structure and gating of the MtrE FA19 appears to be radically different from the previously reported FA136 (4).


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**12**

## The nasal microbiome and Staphylococci


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*Staphylococcus aureus* is a major opportunistic pathogen, commonly colonizing the anterior nares. The nasal microbiota is probably the most important determinant of *S. aureus* colonization could be a potential risk factor for *S. aureus* infection but also a source of novel anti-bacterials or commensals that could compete against *S. aureus*.

*S. aureus* colonizes the human nose from infancy throughout adulthood, but the overall composition of the nasal microbiota changes especially in the first year. During adulthood the nasal microbiota appears to be relatively stable even though we know that there is a certain degree of dynamics.

Lately, there has been great focus on coagulase negative staphylococcus species, particularly *S. lungdunensis*, but also other CoNS that possess the ability to inhibit *S. aureus* growth in a number of ways. My group has investigated the staphylococcal communities of the nose, and I will present our recent findings and relate these to the current knowledge of the nasal microbiota.

**13**

## Unveiling Microbial Diversity And Activity In Cystic Fibrosis Sputa Using Microbial Metatranscriptomics


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Cystic fibrosis (CF) is the most common life-shortening inherited condition in people of European descent, affecting between ~80,000 people globally. CF pathogenesis is most prominent in the airways, where it causes the production of thick, tenacious mucus that provides ideal conditions for microbial pathogens to persist and thrive. Although metagenomics has shown promise with other clinical sample types (e.g. faeces), the CF microbiome has proven particularly challenging due to high (~99%) human DNA contamination, which overwhelms the microbial signal. Here, we used microbial metatranscriptomics (MMT) to examine the polymicrobial population of CF sputa from four Australian adult CF patients. MMT involves extracting total RNA, followed by removal of human mRNA and rRNA, leaving predominantly enriched bacterial mRNA and rRNA for sequencing. Unlike metagenomics, MMT provides an accurate snapshot of the ‘active’ polymicrobial population (i.e. no sequencing of residual ‘dead’ cells or reagent DNA contamination), it captures RNA viruses, it presents fewer ethical issues and greater human nucleic acid depletion efficiency, and it can theoretically identify gene expression differences conferring clinically relevant phenotypes for target species of interest (e.g. antimicrobial resistance caused by efflux pump upregulation). Among the four patients, MMT and 16S microbiomic sequencing yielded taxon assignments that were in broad agreement, although MMT had superior resolution; for example, MMT correctly identified miscalled *Burkholderia* sp. as *Pseudomonas aeruginosa*. Consistent with other studies, Gram-negative anaerobic bacteria were abundant, with *Prevotella* (mainly *P. melaninogenica*), and *Veillonella* spp. found in high abundance in all patients, and the pathogens *Stenotrophomonas maltophilia* and *P. aeruginosa* found in two and three patients, respectively. Taken together, we demonstrate the feasibility of MMT for unveiling the ‘active’ polymicrobial populations present in the CF airways. Although MMT is currently an immature method, it holds great promise for accurately characterising the composition, diversity, and function of polymicrobial infections. Future work is needed to assess the value of MMT for identifying antibiotic resistance and informing antibiotic treatment regimens in CF infections.

**14**

## Genomic insights into a totally new disease caused by Streptococcus agalactiae in Southeast Asia

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Singapore suffered the largest reported outbreak of Streptococcus agalactiae (GBS) disease in 2015, associated with the consumption of raw fish. This was shown to be a totally new paradigm for GBS infection, because GBS was previously not known to be foodborne. In subsequent genomic studies, we have discovered that the particular strain of GBS causing that outbreak, ST283, is almost exclusively found in Southeast Asia. It is causing disease in both humans and farmed fish throughout the region. Our results raise the remarkable hypothesis that, in Southeast Asia, GBS actually is predominantly a foodborne disease caused by a single recently emerged clone. Furthermore, this ST283 clone may have emerged due to the new opportunities afforded by large scale intensive aquaculture fish farming, weaving together complex issues about economic development, international trade, healthcare access, and infectious disease.

**15**

## Rapid transmission and replacement of diverse *Streptococcus pyogenes* isolates along household and community networks in rural Indigenous communities

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Superficial infections with *Streptococcus pyogenes (S. pyogenes*), such as pyoderma and pharyngitis, can lead to invasive diseases, chronic renal/cardiac diseases, and early mortality. Prevalence of *S. pyogenes* infections in many remote Indigenous communities in the Northern Territory (NT) can be as high as 30-40% at any one time. Efforts to control superficial GAS infections and their sequelae in Indigenous populations have been hampered by an incomplete understanding of transmission dynamics and population biology.

We utilised data collected during a longitudinal household study conducted in two geographically-separated remote communities of the NT from 2003-2005. Household were selected based on a known history of rheumatic heart disease. Houses were visited on a monthly basis to screen individuals for sore throat and pyoderma. *S. pyogenes* isolated from skin and throat were subject to whole genome sequencing which was used to determine strain relatedness and define migration of strains throughout households and the communities.

A total of thirty-four genotypes were defined with each genotype forming distinct, closely related subgroups when compared to global isolates of the same *emm/*MLST type. Each genotype was only isolated for a limited amount of time within a community (<6 months). The migration of strains within and between households was investigated using pairwise comparisons. Potential transmissions between individuals was established by isolation of the same genotype at the same period in time or subsequent month with a pairwise SNP distance of <4 SNPs. Using these thresholds >100 within and between household transmission events were predicted.

These data demonstrate the *S. pyogenes* population in rural indigenous communities in the NT is highly diverse but comprises of distinct sub-lineages in comparison to global isolates. Migration of stains in these communities is rapid and genotype replacement occurs frequently. These factors need to be accounted for when implementing control strategies and informing policy design, such as vaccination, to reduce the burden of *S. pyogenes* mediated disease in rural settings.

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**16**

## Transcriptomic, proteomic and functional analysis of the plasmid pCS1-1 during spore formation in *Clostridium sordellii*

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Clostridial plasmids have been shown to carry antibiotic resistance determinants and virulence factors, resulting in the ability of these mobile genetic elements to contribute to pathogenesis. Despite many clostridial strains carrying plasmids, the role of plasmids in sporulation has not been

studied. Using *Clostridium sordellii* as our model spore-forming organism, we have focussed on the toxin-encoding conjugative plasmid, pCS1-1, to investigate if plasmids contribute to, or are influenced by, spore formation.

RNA-Seq analysis on *C. sordellii* strain ATCC9714 identified that the expression of most pCS1-1 genes were downregulated during spore formation, compared to vegetative cell growth; however, three genes were upregulated during sporulation. One of these genes, *asrA*, was insertionally inactivated and the resultant mutant characterised. The plasmid-encoded *asrA* gene influences the temporal progression of sporulation, with the mutant displaying a delayed sporulation phenotype.

A proteomic analysis of mature *C. sordellii* spores was also performed. Purified spores were cryomilled to disrupt their integrity, and mass spectrometry was used to identify the pCS1-1 proteins that contribute to the mature spore proteome. Five of the potential 89 proteins predicted to be encoded by pCS1-1 were found to be present in spores. The function of these plasmid-encoded proteins identified in our proteomics study is currently under investigation.

It has previously been assumed that while plasmids are important during vegetative growth, they are not active or relevant during the process of spore formation. Our results have shown, through a combination of transcriptomic and proteomic analyses, that plasmid-encoded elements are active during sporulation. In addition to this, we have demonstrated genetically that *asrA* on pCS1-1 contributes to the progression of sporulation. This is the first time that a plasmid-encoded gene has been shown to modulate the sporulation process in any spore-forming bacterium. This work supports the need for further research into the role of plasmids throughout all life stages of the clostridia.

**17**

## Revealing the pathogen-specific RNA-binding proteome using UV-crosslinking

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RNA-binding proteins (RBPs) are key players in transcription, translation, and gene regulation in all organisms. High-throughput analysis of the RBPome has shown a surprisingly large number of RNA-protein interactions in model eukaryotes. In bacteria, RNA-binding proteins play critical roles in regulating gene expression and are required for pathogenesis. Until recently, all high-throughput studies to identify RNA-binding proteomes (RBPome) relied on purification of polyadenylated RNAs, and are not suitable for studying bacterial transcripts that lack a poly(A) tail. We have used a new technique termed total RNA-associated protein purification (TRAPP) to capture the RBPome of enterohaemorrhagic *E. coli* (EHEC). This method uses UV-crosslinking, silica beads, and organic extraction to selectively enrich crosslinked RNA-protein complexes. TRAPP identified 264 proteins that were at least twofold enriched in UV-crosslinked cultures and were enriched for proteins with known RNA-binding motifs. Of these, 28 were located on the pathogenicity islands of EHEC. The RNA-binding capabilities of a subset of proteins were assessed by radiolabelling the RNA 5' ends of UV-crosslinked, affinity purified RNA-protein complexes. This demonstrated that 4 of 5 putative RBPs were able to bind RNA *in vivo*. EspY2 was found to bind RNA and is a type 3 secretion system effector protein that is translocated into host cells. Mutation of EspY2 has previously been shown to result in decreased host colonisation. Our results suggest that EspY2 may be a novel example of a bacterial RNA-binding protein that is injected into host cells to promote colonisation. Ongoing experiments are looking to identify the specific RNA targets of EspY2 *in vivo*.

**18**

## The role of glutathione in *Streptococcus pneumoniae* copper resistance at the host-pathogen interface.

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*Streptococcus pneumoniae* (the pneumococcus) is a significant global pathogen, responsible for more than one million deaths each year, primarily in the developing world. As a strictly host-adapted pathogen, the pneumococcus is beholden to the host environment which constitutes its extracellular milieu. As such, essential homeostatic pathways, such as those involved with metal ion homeostasis, have been evolutionarily tuned by the metal stresses encountered at the host-pathogen interface. This includes a high affinity copper efflux pathway, CopA, thought to resist copper intoxication during infection.

Here, we show that during systemic infection, murine tissue copper concentrations increase in numerous niches. However, an isogenic D39Δ*copA* strain does not show significant differences in bacterial load in these niches compared to the wildtype. Interestingly, our *in vitro* analyses show that *S. pneumoniae* does not modulate copper uptake, but rather accumulates copper relative to the environmental abundance. This suggests that, during infection, increased intracellular copper is being buffered within the cytoplasm, possibly on glutathione, to prevent copper toxicity. We therefore investigated a D39Δ*copA*Δ*gshT* strain (lacking copper efflux and glutathione) in *in vitro* growth assays, THP-1 macrophage survival assays and in a murine infection model. Growth assays show that D39Δ*copA*Δ*gshT* was hyper-susceptible to copper stress, indicating a central role for glutathione in copper buffering. This increased sensitivity to copper was also observed in the infection model, with D39Δ*copA*Δ*gshT* less viable in multiple niches compared to the single Δ*copA* and Δ*gshT* mutants. Interestingly, even in macrophages, where it is postulated that invading pathogens are directly exposed to high concentrations of copper, survival of D39Δ*copA* was equivalent to wildtype, with decreased survival observed only for D39Δ*gshT* and D39Δ*copA*Δ*gshT*.

Collectively, these data show that host-imposed copper stress is well-tolerated by the pneumococcus providing glutathione is available. These findings also have significant implications for copper-based antimicrobial measures, which are likely to have limited efficacy against *S. pneumoniae* while glutathione can be actively acquired.

**19**

## Copper and zinc resistance mechanisms contribute to Group B streptococcal virulence and pathogenesis

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Group B streptococcus (GBS; *Streptococcus agalactiae*) is a globally disseminated opportunistic pathogen that causes sepsis, meningitis, pneumonia and soft-tissue infections in healthy or immunocompromised adults. GBS is the leading cause of neonatal infections with 0.6 cases/1000 live births and 6% mortality rate in Australia. GBS is usually a commensal of the genitourinary tract but colonizes a variety of niches within the body and is capable of residing inside host cells in the intracellular environment. Antimicrobial metal ions such as zinc or copper are concentrated within host cells as an immune response to kill invading pathogens. Bacteria possess import and efflux mechanisms to fine-tune Zn or Cu levels, such that free ions remain diminishingly low inside the bacterial cytoplasm. We recently elucidatedmechanisms of Zn and Cu resistance in GBS and defined the regulation of efflux by metal ion responsive transcription factors. By combining metal-ion survival assays, mutational and transcriptional analyses, and *in vitro* and *in vivo* disease models, we show that resistance to Zn and Cu significantly alters the ability of GBS to survive within the host and cause disseminated infection. In addition, our data show that hypervirulent GBS isolates are more susceptible to killing by Zn or Cu. Together, our observations highlight novel aspects of Zn and Cu resistance strategies in GBS that could be targeted in future therapeutic approaches for GBS treatment and prevention.

**20**

## Role of Integrative Conjugative Elements in the evolution of the kiwifruit pathogen *Pseudomonas syringae* pv. *actinidiae*

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Horizontal gene transfer is a major force driving evolution in prokaryotes and has played a direct role in the evolution of pathogenicity and virulence in numerous bacterial pathogens. We have identified a large family of 'integrative and conjugative elements' widely distributed among plant-associated *Pseudomonas* spp. (PsICEs), including P. *syringae*. While PsICEs had diverged at the nucleotide level, they shared the same integration site and a conserved syntenic set of backbone genes interspersed with various accessory regions. Accessory regions included genes for resistance to heavy metals, type 3 secretion system effectors but the most common element is the transposon Tn*6212*. The kiwifruit pathogen *P. syringae* pv. *actinidiae* (*Psa*) was responsible for a devastating agricultural pandemic in 2008. Sequencing of globally dispersed isolates revealed three divergent PsICEs lineages carrying Tn*6212* were independently acquired by *Psa* pandemic strains, suggesting this transposon might confer a selective advantage to its host. Tn*6212* provided a competitive advantage to *Psa* NZ13 when grown in succinate, malate, fumarate and citrate as only carbon sources. RNA-seq experiments demonstrated Tn*6212* can manipulate expression of both core-chromosomal and PsICE genes. In *Psa*, the PsICEs were also responsible for the acquisition of resistance to copper, the most common antimicrobial compound used in agriculture. Interestingly, while copper resistance provided a selective advantage, the acquisition of the new element did not impose any competitive fitness cost to *Psa* NZ45. We have described a novel family of ICEs and showed how their accessory genes can contribute to the fitness of *Psa*. PsICEs, having control over both vertical and horizontal modes of transmission, minimizing costs for host cell while introducing new beneficial traits, are potent vehicles of microbial evolution.

**21**

## The role of zinc acquisition and zinc tolerance in Group A Streptococcal infection

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Zinc is an important nutrient in biology. Zinc deficiency is associated with increased susceptibility to bacterial infections. Since the innate immune system can utilise both zinc starvation and toxicity strategies to combat infections, therefore, bacterial pathogens need strict control of zinc homeostasis. Here, we investigate the role of zinc import and export in protection of *Streptococcus pyogenes* (Group A *Streptococcus*; GAS), a Gram-positive bacterial pathogen responsible for a wide spectrum of human diseases, against challenge from host innate immune defence. In order to determine the role of GAS zinc import and export during infection, we utilized the zinc import (Δ*adcA/AII*) and export (Δ*czcD*) deletion mutants in competition with wild-type in both *in vitro* and *in vivo* virulence models. We demonstrate that nutritional immunity is deployed extracellularly while zinc toxicity is utilized upon phagocytosis of GAS by neutrophils. We also show that lysosomes and azurophilic granules in neutrophils contain zinc stores for use against intracellular pathogens. We identified that the stage and site of infection differ in the use of zinc as a control strategy.

**22**

## *Acinetobacter baumannii* employs multiple pathways for zinc and cadmium efflux

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*Acinetobacter baumannii* is a Gram-negative human pathogen associated with significant morbidity and mortality. The WHO has placed *A. baumannii* as the top critical pathogen in need for novel antimicrobial therapies due to the emergence of carbapenems resistant isolates. Metal ions, such as zinc, have been recognised as important antimicrobials to control bacterial infections. Thus, resistance to metal intoxication is crucial for the success of many pathogenic bacteria. *A. baumannii* is known to harbour an extensive repertoire of metal ion efflux systems, none of which have been functionally characterised. Here, we investigated the role of membrane transport systems in *A. baumannii* zinc resistance. Our analyses of transposon mutant *A. baumannii* strains revealed a role for the resistance nodulation division (RND) transporter CzcCBA in zinc resistance. This was determined by supplementing the mutant strain *czcA*::T26 with zinc, and examining the impact on growth and metal accumulation compared to the wild-type strain. The significance of this pathway was then investigated using a zinc-deficient murine infection model. This revealed that *A. baumannii* resistance to zinc stress was important in the spleen, as indicated by the reduced bacterial burden of the *czcA*::T26 strain by comparison to the wild-type strain. Our studies also identified an additional zinc resistance pathway, the cation diffusion facilitator (CDF) protein CzcD. We then investigated the contribution of these pathways in resistance of other transition metal ions. This revealed that CzcCBA contributed to cadmium resistance, and while CzcD did not provide resistance against cadmium, we identified a distinct CDF transporter, CzcE, that is crucial for *A. baumannii* survival in cadmium stress. Collectively, these analyses provide novel insights into the metal ion resistance mechanisms of *A. baumannii,* and the niches in which metal ion tolerance are important during infection.

**23**

## Marmoset model of melioidosis

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Not available

**24**

## Protein *O*-glycosylation is conserved in *Burkholderia* species and results in the generation of *O*-glycosylation glycan specific antibodies during human infections

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*Burkholderia is a* ubiquitous Gram-negative genus comprised of genetically and phylogenetically diverse species. Although most members of this genus do not cause disease, some species (including *B. cenocepacia*, *B. multivorans* and *B. pseudomallei*) are now recognised to cause infections within immunocompromised groups and nosocomial cohorts. Previously we demonstrated the presence of an O-linked protein glycosylation system within *B. cenocepacia* which was required for virulence (1), yet did not define the cluster required for the generation of the O-linked glycan. Within this work, we identify the O-glycosylation (ogc) cluster necessary for synthesis, assembly, and membrane translocation of the lipid-linked O-glycan, in addition to determining the chemical structure of this trisaccharide. Surprisingly we find the ogc cluster is conserved in the Burkholderia genus and confirm the production of glycoproteins with similar glycans in *B. thailandensis*, *B. gladioli* and *B. pseudomallei* isolates. Further, we show that absence of protein O-glycosylation severely affects bacterial fitness and accelerates *B. cenocepacia* clearance in a larvae infection model. Proteomic analysis of glycosylation mutants within *B. cenocepacia* confirms widespread alterations within virulence associated proteins, and defects in cellular processes, supporting the importance of O-glycosylation for bacterial fitness. Finally, we demonstrate that the conservation of protein glycosylation and the ogc results in patients infected with *B. cenocepacia*, *B. multivorans*, *B. pseudomallei*, and *B. mallei* developing O-glycan specific antibodies. Together, these results highlight the importance of general protein O-glycosylation in the biology of the Burkholderia genus and its potential as a target for developing novel inhibition and immunotherapy approaches to control Burkholderia infections.

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**25**

## Acute *Clostridioides difficile* gastrointestinal infection induces systemic disease symptoms, in a toxin and time dependent manner

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*Clostridioides difficile* is the most common identifiable cause of infectious antibiotic-associated diarrhoea in developed countries, with disease mediated by the action of the two major toxins TcdA and TcdB, and a third toxin, CDT. In severe cases, disease can be fatal, with the patient presenting with severe colonic damage and, less frequently, with systemic disease complications. Systemic disease in animal models has also been described, with thymic disorganisation and apoptosis occurring during severe disease in mice. Here, we examine this disease phenotype and show that a ribotype 027 strain of *C. difficile* causes toxin-dependent thymic atrophy and disorganisation in mice. Disease damage results in reduced thymic size, disorganisation of the thymic cortex and medulla, as well as a reduction of the $CD4^+CD8^+$ thymocyte population and proportional changes in the single positive $CD4^+$ or $CD8^+$ naïve T cell populations. We also showed that thymic atrophy is not restricted to a single strain or genetic background of *C. difficile,* with strains from different ribotypes able to induce disease and thymic atrophy. This atrophy appeared to be linked to the severity of colonic damage, as thymic atrophy was absent in mice that were infected with strains that did not induce severe colonic damage, suggesting toxin-mediated enteric damage induces the leakage of gut contents, including toxins, into circulation, promoting systemic disease and inflammatory responses. Administration of bezlotoxumab (Zinplava), a monoclonal TcdB therapeutic, rescued mice from systemic disease, preventing thymic atrophy and $CD4^+CD8^+$ thymocyte depletion, as well as increasing mouse survival, when compared to treatment with vehicle alone (PBS). As the thymus has such a crucial role in T cell production and immune system development, these findings may have important implications in relapse of *C. difficile* disease and impaired immunity during *C. difficile* infection.

**26**

## Investigating the role of a novel cell division protein, YtfB, in eukaryotic cell adhesion

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Many proteins are assigned a function based on predicted computational homology. However, characterisation of function based solely on homology searches may overlook functions under specific environmental conditions, or the possibility of a protein having multiple functions. In this study we investigated the function of YtfB, a protein originally identified in a genome-wide screen to cause inhibition of cell division when overexpressed[1], and which has been recently demonstrated to localise to the *Escherichia coli* division site with some degree of glycan specificity[2]. Interestingly, YtfB also shows homology to the virulence factor OapA from *Haemophilus influenzae*, which is important for adherence to epithelial cells, indicating the potential of additional function(s) for YtfB. Here we show that *E. coli* YtfB binds to chitosan and di-mannose glycans with high affinity. The loss of *ytfB* results in a reduction in the ability of the uropathogenic *E. coli* strain UTI89 to adhere to kidney cells, but not to bladder cells, suggesting a specific role the initial adherence stage of ascending urinary tract infections. Additionally, the Δ*ytfB* mutant is outcompeted for growth by the wild type in human urine, whilst loss of YtfB results in hypermotility. Taken together, our results suggest a role for YtfB in the switch of a motile to a sessile lifestyle in the environment of the urinary tract, which may be additional, or complementary, to its role in cell division, and highlights the importance of understanding protein function in the context of different environmental conditions.

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**27**

## Formation of the spacious *Coxiella*-containing vacuole depends on host transcription factors TFEB and TFE3.

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*Coxiella burnetii*, the etiological agent of the zoonotic infection Q fever, is an obligate intracellular bacterial pathogen that replicates inside the lysosome-derived *Coxiella*-containing vacuole (CCV). The CCV maintains the hydrolytic and acidic nature of the host lysosome despite *C. burnetii* directing the massive expansion of this compartment to accommodate the replicating pathogen. To establish this unique replicative niche, *C. burnetii* utilizes the Dot/Icm type IV secretion system to translocate approximately 150 effectors into the host cell that modulate various cellular processes. In order to characterize the host-pathogen interactions that occur during *C. burnetii* infection, SILAC based proteomics was

performed to examine changes in the host proteome during infection of THP-1 cells. This proteomic analysis showed that during *C. burnetii* infection, the abundance of many proteins involved in host cell autophagy and lysosome biogenesis was increased. Given this finding, the role of host transcription factor's TFEB and TFE3, that regulate the expression of a network of genes involved in autophagy and lysosomal biogenesis, was examined. Three days post-infection with *C. burnetii* both TFEB and TFE3 were activated as demonstrated by their trafficking from the cytoplasm into the nucleus. Nuclear to cytoplasmic ratio of TFEB and TFE3 was measured using a custom ImageJ macro. The nuclear translocation of these transcription factors appears to be controlled by *C. burnetii* as blocking bacterial translation with chloramphenicol led to TFEB and TFE3 movement back into the cytoplasm. siRNA silencing of *tfeb* and *tfe3* demonstrated that these transcription factors are important for CCV expansion suggesting they play a role in the intracellular success of *C. burnetii*.

**28**

## Spreading antibiotic tolerance in the Enterobacteriaceae: a novel toxin antitoxin system in major resistance plasmids confers antibiotic and heat tolerance and promotes biofilm formation.

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Toxin-antitoxin (TA) systems were initially discovered as plasmid addiction systems on low-copy-number plasmids. Thousands of TA loci have since been identified on chromosomes, plasmids and mobile elements in bacteria and archaea with diverse roles in bacterial physiology and in maintenance of genetic elements. Here, we identified and characterised a plasmid mediated type II TA system in Enterobacteriaceae as a member of the ParDE super family. TA-finder was used to identify TA systems in a conjugative IncI antibiotic resistance plasmid. Blast, MEGA, and PSIPRED software were used to elucidate distribution, identity and secondary structures. Plasmid maintenance, stress tolerance and biofilm production were assessed. This TA system (hereafter, ParDE$^{I}$) is distributed among IncI and IncF-type antibiotic resistance and virulence plasmids found in avian and human-source *Escherichia coli* and *Salmonella.* ParDE$^{I}$ is a plasmid stability and stress response module that increases tolerance of aminoglycoside, quinolone and β-lactam antibiotics in *E. coli* by ~100-1000-fold, and thus to levels beyond those achievable in the course of antibiotic therapy for human infections. ParDE$^{I}$ also confers a clear survival advantage at 42 °C and expression of the ParE$^{I}$ toxin *in trans* induces the SOS response, inhibits cell division and promotes biofilm formation. The spread of plasmid-borne high-level antibiotic tolerance is clearly a major threat and likely to be an important factor in the success of the IncI and IncF plasmids which carry it and the important pathogens in which these are resident.

**29**

## A pipeline for bacteriophage (phage) discovery, characterization and pre-clinical evaluation.

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The rise in resistance to existing antimicrobial drugs is a major global health threat. These antimicrobial resistant (AMR) infections are caused by bacteria and fungi of various species, collectively referred to as "superbugs". *Klebsiella* is now established as a pathogen with high rates of AMR and concurrent mortality in humans, and work in our lab is directed towards reversing the evolution of AMR phenotypes in *Klebsiella*. Approaches to combat AMR that obviate the need for new drugs include the re-engineering/re-purposing of existing drugs, and phage therapy. Bacteriophage (phage) are viruses that selectively kill bacteria, and have proven effective in curing infections caused by superbugs. We have been working in two areas of importance in phage therapy: the discovery of diverse phage for use in producing "phage cocktails" for therapy, and detailed means for characterization of new phages towards adapting them as biologics. Using environmental water samples from around the world, we discovered phage with diverse tail structures targeting *Klebsiella* by diverse means. Cryo-electron microscopy is being used to understand the tail structures, and work towards developing biologics isolated from phages, including robust enzymes that degrade bacterial biofilms, are being pursued.

**30**

## Takes two to tango: characterizing the interactions between *Klebsiella pneumoniae* and its phages.

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Multidrug resistant (MDR) *Klebsiella pneumoniae* (*Kp*) CG258 clones, refractory to routine antibiotic treatment, are considered a particularly serious clinical threat by the WHO as cause of infection outbreaks in humans worldwide [1]. Lytic bacteriophages (phages) are natural viral predators of bacteria and effective agents of bacterial clearance that may be used against MDR species (phage therapy) as alternatives or adjunctives to antibiotics [2]. However, implementation of phages as routine human therapeutics is hindered by poor translation of successful *in vitro* outcomes to *in vivo* applications, related to our yet limited understanding of complex bacteria-phage dynamics, that restricts *a priori* prediction of therapeutic efficacy [2,3]. In our work, we aim to explore these important potential barriers to the widespread and effective deployment of phage therapy for bacterial pathogens, using MDR *Kp* CG258 as our model pathogen. We have characterised a number of phages that specifically destroy the most resistant and virulent subtype of *Kp* CG258 (*Kp* ST258), and particularly the clade that dominates in Australian hospitals (ST258 clade 1) [4], and identified candidate tail fiber genes responsible for target specificity using *Kp* ST258 clade 1 capsular variants and

porin mutants. Co-incubation in liquid culture of target *Kp* and phages, with different selective specificity, produced phage-resistant mutants with different morphology (mucoidy levels). Colonies were sequenced (Illumina NextSeq) and comparison with the parent strain showed diverse patterns of genomic variants (some shared, some unique), and multiple mutant types. We will next characterize attachment mechanisms for selected phage-*Kp* pairs in order to determine whether the primary selective pressure for the antagonistic changes (phenotype modulation/genetic modification) in response to virulent phage attack, is linked directly to phage receptor specificity, with crucial implications for the development of effective personalized phage treatments.


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4. Venturini et al (2019) bioRxiv 628339; doi: https://doi.org/10.1101/628339.


**31**

## Characterization of an equine *Klebsiella pneumoniae* infection using *de novo* isolated bacteriophages


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*Klebsiella pneumoniae* is a frequent cause of opportunistic, often severe, infections in mammals, including sepsis, pneumonia and mastitis, which are commonly treated with antibiotics. *K. pneumoniae* infection in horses can lead to potentially life-threatening haemorrhagic pneumonia with high treatment costs, particularly for infections refractory to first-line antibiotics, where use of amikacin is required (Estell et al., 2016). The rise in multidrug resistance in *K. pneumoniae* is therefore a serious veterinary concern (Ewers et al., 2012). Early identification of infective types and adjunctive therapies to antibiotics could prove important aids for timely and effective treatment. Use of bacteriophages for pathogen tracking (Sechter et al., 2000) and as alternative antibacterial agents (Khan Mirzaei & Nilsson, 2015) is being actively pursed in the era of multidrug resistant superbugs, and better understanding of the dynamic interactions between pathogenic bacteria, phage and the mammalian host is crucial for successful therapeutic outcomes.

In this study, we tracked and characterised *K. pneumoniae* isolated from bronchial aspirates of a horse with severe respiratory infection using *de novo* isolated bacteriophages. Predominant *K. pneumoniae* clones in two bronchial specimens, collected five days apart, were isolated by standard microbiology and typed based on cross-susceptibility to bacteriophages raised *de novo* against each, and according to their antibiotic resistance phenotype. Bacterial isolates with unique profiles were further characterised by pulsed field gel electrophoresis (PFGE) and whole genome sequencing (WGS). Multiple distinct *K. pneumoniae* strains were identified in these specimens with unique PFGE profiles. Bacteria-phage interactions (susceptibility profiles) allowed for early discrimination between different types and showed that predominant clones changed over the course of infection, as confirmed by WGS analysis and antibiotic resistance profiles. Use of bacteriophage susceptibility testing can be an additional rapid and cheap method for tracking *K. pneumoniae* infection.

**32**

## Genetic diversity and distribution of filamentous prophage in *Neisseria*


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A filamentous bacteriophage termed the Meningococcal Disease Associated (MDA) phage is associated with *Neisseria meningitidis* (NM) clades which cause invasive meningococcal disease. MDA ϕ improves mucosal colonization of the nasopharynx by meningococci thus increasing the probability of bloodstream invasion associated with meningococcal carriage. We recently recovered a *Neisseria gonorrhoeae* (NG) isolate (ExNg63) from a rare case of gonococcal meningitis. Whole genome sequencing revealed the isolate possessed a region with 90% similarity to MDA ϕ found in NM. This is the first indication that MDA-like prophages may not be restricted to NM. To understand the genetic diversity and distribution of MDA-like prophages, we examined the distribution, prevalence and genetic diversity of filamentous phages in *Neisseria*. Closed genomes of 44 NM, 28 NG and 19 commensal *Neisseria* species were collected from the NCBI database and BIGSdb and were examined for presence of filamentous prophages. Maximum likelihood phylogenetic trees were constructed using MEGA7 while heirBAPS was used to define genetic population groups. BEAST software was used to construct timed phylogeny of the MDA genomes. One hundred and sixty filamentous prophages were detected in the dataset and population structure analysis using heirBAPS revealed the putative gonococcal MDA-like prophages and a putative MDA-like prophage in *Neisseria lactamica* (NL) formed a structure group with meningococcal MDA ϕ. However, only 7.5% of gonococcal isolates available at BIGSdb possessed a complete or partial MDA-like sequence compared to 46% of meningococcal isolates suggesting acquisition of MDA-like prophages is more restricted in NG. BEAST time measured phylogeny of the MDA genomes indicated the most likely source of MDA in NG was from NM serogroup B that could be acquired through natural transformation. These data suggest that prophages similar to the meningococcal MDA ϕ are present in NG and NL and more work is required to determine whether MDA-like prophages are active and can act as accessory colonization factors in these species.

**33**

## Serogroup W clonal complex 11 meningococci from Western Australia have increased invasive potential compared to other hyperinvasive lineages.

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Serogroup W *Neisseria meningitidis* isolates from clonal complex 11 (MenW:cc11) are responsible for outbreaks of invasive meningococcal disease worldwide. In Western Australia, two distinct lineages of MenW:cc11 isolates have emerged since 2016, Cluster A and Cluster B, the latter of which is penicillin-resistant and was responsible for 81% of all MenW cases in WA since 2018.This study characterised the virulence of three Cluster A and three B representatives by investigating the expression of major known virulence determinants and comparing attachment and invasion into Detroit 562 epithelial cells. Immunoblots to detect expression of Opa and PilE surface antigens showed that these determinants were not phase-variable in all six MenW:cc11 isolates analysed. No differences in the genotype of the loci encoding the minor adhesins NadA, NhhA, fHbp, and App were observed among MenW:cc11 isolates, while the *opcA* locus was not detected in all isolates. Bioinformatics analysis of the whole genome revealed that all isolates were predicted to express the L2/L4 immunotype lipooligosaccharide which has a terminal lacto-*N*-neotetraose (LNT) moiety. Tricine SDS-PAGE showed that all isolates had a LOS with a mass of 4.6 kDa consistent with a L2/L4 LOS, but only Cluster A isolates reacted with the 3F11 monoclonal antibody (MAb) that detects LNT. All three Cluster B isolates exhibited a 3F11-negative phenotype which is often the result of blocking MAb binding by terminal sialylation of the LNT. A comparison of three MenW:cc11 strains with a cc8 strain NMB found no significant difference in their ability to colonise Detroit 562 cells. However, two strains belonging to Cluster A displayed a significant 21.0- and 16.0-fold increase in invasion, respectively, compared to strain NMB. In conjunction with previous work, these results demonstrate a wide range of invasive potential amongst meningococcal lineages even when the expression of known virulence factors are accounted for. The factors contributing to the increased invasive potential of MenW:cc11 isolates from WA have yet to be identified.

**34**

## Zinc homeostasis in *Klebsiella pneumoniae*

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*Klebsiella pneumoniae* is a Gram-negative bacterial pathogen, most commonly associated with respiratory and nosocomial infections. It is also an ESKAPE pathogen and a WHO priority pathogen due to high levels of antibiotic resistance. As such, alternate methods for control of pathogenicity are highly desirable. Zinc is a first-row transition metal ion that is essential for numerous biological functions in all forms of life. Nevertheless, it can exert significant toxicity in excess. Accordingly, bacterial cells tightly regulate cytoplasmic zinc abundance to ensure sufficiency for physiological functions and preventing excess accumulation that can lead to protein mismetallation. It logically follows that interference with these pathways may provide novel avenues for antimicrobial therapies.

Zinc homeostasis remains poorly defined in *Klebsiella pneumoniae*. However, model Enterobacteriacae, namely *Escherichia coli* and *Salmonella enterica*, have been extensively studied with respect to zinc homeostasis. In these bacteria, zinc homeostasis is mediated by a number of proteins, including the zinc responsive regulator Zur, the Znu and Zup importer systems and Znt, Zit and Mdt exporter systems. Here, we report an investigation into the zinc homeostatic mechanisms of *K. pneumoniae* strain AJ218. This was addressed by examining phenotypic growth and how it was impacted in zinc restricted and replete conditions. Transcriptional analyses identified genes associated with zinc starvation and intoxication. We then generated mutant strains deficient in the putative *zur*, *znuA* and *zntA* genes and assessed the impact on resistance to zinc stress. These data provide a foundation for further studies of zinc homeostasis and its role in the physiology of this priority bacterial pathogen.

**35**

## Elucidating the Zn(II)-binding mechanism of *Streptococcus pneumoniae* AdcAII

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*Streptococcus pneumoniae* is a globally significant human pathogen responsible for more than a million deaths annually. To colonise and persist within the host, the bacterium must acquire the transition metal ion zinc [Zn(II)], which is poorly abundant in the host environment. In *S. pneumoniae*, Zn(II) import is facilitated by the ATP-binding cassette transporter, AdcCB, and two Zn(II)-specific solute binding proteins (SBPs), AdcA and AdcAII. Although both proteins deliver Zn(II) to the AdcCB transporter, AdcAII has a greater role during initial infection and survival in response to Zn(II) starvation. Despite this, the molecular details of how AdcAII selectively acquires Zn(II) remain poorly understood. To date, our understanding of the Zn(II)-binding mechanism has been based solely on the crystal structure of Zn(II)-bound AdcAII, with an open, metal-free conformation remaining refractory to crystallographic approaches. As a consequence, the conformational changes that occur within AdcAII upon Zn(II)-binding remain unknown. Here, we overcame this challenge by individually mutating each of the four Zn(II)-coordinating residues and

performing structural and biochemical analyses on the variant isoforms. Structural analyses revealed specific regions within the protein that underwent conformational changes via their direct coupling to each of the metal-binding residues. Quantitative metal-binding, metal ion affinity analyses and phenotypic assays revealed that two of the four coordinating residues had essential contributions to the Zn(II)-binding mechanism of AdcAII. Intriguingly, only one of these residues had a direct role in structural rearrangements within AdcAII. These analyses also revealed that AdcAII could interact with other first-row transition metal ions, in contrast to AdcA. Collectively, our structural, biophysical and microbiological data indicate that AdcAII employs a distinct mechanism of metal binding to other Zn(II)-specific SBPs. Elucidation of this mechanism will provide the structural and biochemical data required for future antimicrobial design strategies.

**36**

## Trehalase Activity Supports *Burkholderia pseudomallei* Virulence and External Survival

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*Burkholderia pseudomallei* exists as a normal resident of tropical and sub-tropical soils worldwide, often infecting hosts via incidental exposure to disturbed soils in the wake of natural disasters and severe weather events. The resulting disease Melioidosis constitutes a life-threatening challenge, as *B. pseudomallei* aggressively colonises most host tissues contributing to difficulties in clinician recognition and treatment efficacy. Understanding what supports *B. pseudomallei* expansion within the host could provide new targets for anti-virulence drug design. The enzyme effector trehalase (*treA*) is used by *B. pseudomallei* for the hydrolysis of the disaccharide trehalose, a molecule found in the environment and host tissues. Interestingly, trehalose metabolism has been demonstrated to significantly impact virulence in multiple melioidosis host models[1]. This work aims to further define the participation of *treA* in both external survival and virulence, with the purview that novel inhibitors targeted against *treA* could assist therapeutic intervention. To re-establish the phenotype demonstrated in prior studies, a new unmarked deletion mutant (Δ*treA*) was constructed in our laboratory *B. pseudomallei* K96243. This mutant was assessed for its capacity to replicate wild-type *B. pseudomallei* infectivity within an *in vitro* macrophage model, in addition to static biofilm formation, and growth on trehalose. In comparison to wild-type *B. pseudomallei*, Δ*treA* was unable to grow when provided with trehalose as the sole carbon source ($p$<0.0001). This was fully restored upon complementation with *B. pseudomallei* K96243 *treA*. Furthermore, Δ*treA* exhibited significantly diminished biofilm formation capacity ($p$<0.001) after 72-hours of continuous growth. Using novel trehalase inhibitors, we now seek to replicate these *in vitro* effects with concurrent investigation into the localization of trehalase protein within the *B. pseudomallei* microenvironment, via tagged complementation. Our preliminary investigations reveal the potential utility of pursuing trehalase as a novel target for therapeutic intervention. By interfering with trehalase, prospective inhibitors may constrain the rapid growth of *B. pseudomallei* in patients and reduce the outright virulence permitted by normal metabolic activity.

1Vanaporn, M., Sarkar-Tyson, M., Kovacs-Simon, A., Ireland, P.M., Pumirat, P., Korbsrisate, S., Titball, R.W. and Butt, A., 2017. Trehalase plays a role in macrophage colonization and virulence of Burkholderia pseudomallei in insect and mammalian hosts. Virulence, 8(1), pp.30-40.

**37**

## Defining the regulon of WalKR; the only essential two-component system in *Staphylococcus aureus*

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*Staphylococcus aureus* continues to exert significant pressure upon public-health in Australia, both in the clinic and the community. Molecular dissection of systems essential for staphylococcal viability can provide valuable intelligence in the search for new strategies to combat this pathogen. One such system is WalKR; the only essential two-component regulator in *S. aureus*. In addition to a role in maintaining cell wall homeostasis, the *walKR* locus is a hotspot for mutations conferring resistance to last-line antibiotics. Despite the importance of this system its regulon is not well defined, and a robust explanation of its essentiality is yet to be elucidated.

Here, we use a functional genomics approach to interrogate a panel of WalKR 'up' and 'down' mutants, using data from ChIPseq and RNAseq to define a WalR binding motif and characterise the direct regulon of WalKR. In addition to controlling several autolysins involved in cell wall homeostasis, it appears that WalR mediates direct control of genes involved in a variety of other cellular processes, some of which are essential. Subsequently, we employed fluorescence-based promoter reporters, electrophoretic mobility shift assays, and Western blot analysis, to validate our initial observations. Our results provide new insights into the essentiality of WalKR, and ultimately may lead to novel methods for disrupting this system.

**38**

## Epsilon toxin, Mal and multiple sclerosis

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Epsilon toxin, a pore forming toxin produced by *Clostridium perfringens* types B and D strains, is one of the most potent bacterial toxins. The toxin is responsible for the pathogenesis of enterotoxaemia, an often fatal neurological disease of livestock, particularly sheep and goats. A receptor for the toxin has recently been identified as myelin and lymphocyte (MAL) protein. The molecular structures of the monomeric and pore forms of the toxin reveal the mode of action of the toxin and identify how the toxin interacts with MAL. This data has been used to devise a genetic toxoid (Y30A-Y196A-A168F) vaccine. The immunisation of rabbits or sheep with Y30A-Y196A-A168F induced high levels of neutralising antibodies against epsilon toxin which persisted for at least one year. The finding that the toxin recognises MAL has also stimulated work to investigate the possible

role of this toxin in multiple sclerosis (MS), and antibodies to the toxin have been found in the sera of some MS patients. However, evidence for a role for the toxin in the MS is not conclusive.

**39**

## Tyrosine phosphorylation modulates OmpR and H-NS virulence regulation in *Shigella flexneri* and *Escherichia coli*

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In recent times tyrosine phosphorylation has been shown to play a vital role in bacterial virulence, from regulation of capsule in *Streptococcus pneumoniae*, to modulation of T3SS function in *Shigella flexneri*. Furthermore, we and others have shown that tyrosine phosphorylation is more prevalent in bacteria than originally thought, with levels above that seen in eukaryotes. Included amongst proteins tyrosine phosphorylated in *S. flexneri* and *E. coli* are a range of DNA binding transcription factors including the response regulator OmpR and the global repressor H-NS, which are both known regulators of bacterial virulence. We were thus interested to see if tyrosine phosphorylation of these proteins modulated their regulatory ability, and thus their ability to control bacterial virulence.

In order to investigate this, we constructed phosphomimetic and phosphoablative substitutions in *E. coli* and *S. flexneri* on all sites of tyrosine phosphorylation in both OmpR and H-NS. We then investigated whether these substitutions modulated OmpR and H-NS activity, by analysing the level of regulated proteins OmpC and OmpF, as well as bacterial growth. This work suggested that tyrosine phosphorylation of both OmpR and H-NS modulated their regulatory abilities, with dysregulated OmpC/OmpF expression and aberrant growth similar to that seen with a complete deletion of the respective regulator itself. For OmpR, electrophoretic mobility shift assays showed that tyrosine phosphomimetic substitution resulted in a deficiency in the ability of OmpR to bind DNA, illustrating a mechanism for dysfunctional gene regulation.

This work unlocks another layer of complexity behind how bacterial pathogens regulate gene expression, and provides further evidence of the key role that tyrosine phosphorylation plays in bacterial virulence.

**40**

## The macrophage infectivity potentiator protein: Evaluation of a novel target in Gram-negative bacteria for therapeutic intervention.

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*Coxiella burnetii* and *Burkholderia pseudomallei* are Gram-negative intracellular pathogens found in the environment and are the causative agents of Q-fever and melioidosis, respectively. Both diseases are endemic in Australia, are a challenge to diagnose and their treatment is often very difficult. Complicating this further is that currently there is no vaccine available against *B. pseudomallei* and the only available vaccine for Q-fever, Q-Vax, requires pre-vaccination testing due to the risk of severe adverse reactions, limiting its use as a countermeasure to contain outbreaks of this highly infectious organism. Therefore the identification of new drug targets against these organisms is essential.

Macrophage infectivity potentiators (Mips) are proteins that catalyze the folding of proline-containing proteins through their peptidyl prolyl *cis-trans* isomerase (PPIase) activity and have been shown to play an important role in the virulence of several pathogenic bacteria. To this end, the Mip proteins of *C. burnetii* and *B. pseudomallei* exhibit virulence-associated PPIase activity. Our data shows that inhibition of Mip represents a potentially novel target for antimicrobial therapies against intracellular pathogens. Using a protease coupled PPIase assay, we have demonstrated that a selection of pipecolic acid-derived compounds have inhibitory properties against recombinant Mip proteins from *C. burnetii* and *B. pseudomallei*. Importantly, these compounds also inhibit both C. *burnetii* growth in axenic media and intracellular survival *in vitro*, validating these molecules as novel therapeutics. Furthermore these compounds also reduce the cytotoxic effects of *B. pseudomallei* on macrophages demonstrating that these Mip inhibitors have broad-spectrum activity.

**41**

## Phase-variable regulation in *Streptococcus pneumoniae* pathobiology and vaccine development

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*Streptococcus pneumoniae* is the most common cause of bacterial illness worldwide, causing more than 1.5 million deaths annually. *S. pneumoniae* commonly colonises the nasopharynx of healthy individuals asymptomatically. A complete understanding of the mechanism(s) involved in pneumococcal pathogenesis is lacking. Current vaccines against *S. pneumoniae* (PCV-13 and PPSV-23) remain ineffective against untargeted strains. A complete understanding of the pathobiology of *S. pneumoniae* will aid development of more effective vaccines and treatments. Our recent work reports a novel, randomly switching, $N^6$-adenosine DNA methyltransferase (the SpnD39III system) producing six different specificities (alleles A-F). These variants produce six distinct phenotypes via epigenetic regulation of multiple genes; a phasevarion. We sought to characterize the phenotypes of the six SpnD39III alleles by examining the effect of SpnD39II switching to clinically relevant traits such as survival in human blood, biofilm formation, adherence and invasion, and capsule production. We evaluated if SpnD39III allele switching occurred *in vivo* (mice) across different host niches. The effect of SpnD39III phase-variation on current protein vaccine candidate expression was also investigated using RT-qPCR and Western Blot methodologies. Analysis showed that SpnD39III switching influences multiple differences in

phenotype *in vitro*. *In vivo* analysis suggests selection for/against particular SpnD39III in different host niches. Significant differences in putative vaccine gene/protein target expression was also influenced by SpnD39III phase-variation. This data provides a robust understanding of gene regulation and pathobiology influenced by phasevarion switching, and will inform and direct future vaccine development against this major human pathogen.

**42**

## The role of regulatory RNAs in *Acinetobacter baumannii*

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The Gram-negative bacterium *Acinetobacter baumannii* causes life-threatening nosocomial infections and has a near unparalleled capacity to develop multidrug-resistance. Small non-coding RNAs (sRNAs) regulate bacterial physiology, antibiotic resistance, and virulence in many pathogens; however, there has been limited characterisation of these important regulatory molecules in *A. baumannii*. We performed bioinformatic analysis on multiple whole-transcriptome RNA-sequencing datasets and identified more than 40 putative sRNAs that were highly conserved in strains AB5075, AB307-0294, ATCC 17978, and ATCC 19606; several of these putative sRNAs were selected for initial functional characterisation in AB307-0294. Northern blot analyses confirmed the expression and determined the approximate size of these sRNAs, fluorescent primer extension defined the 5' end of each sRNA transcript, and quantitative reverse-transcription PCR was used to assess the expression level of each sRNA at different bacterial growth phases. Deletion mutants and overexpression strains were constructed for each of the sRNA genes. These strains were transcriptionally analysed to identify gene expression changes, relative to expression in wild-type AB307-0294. Small sets of genes were found to be dysregulated in each of the sRNA mutant strains and sRNA overexpression strains and phenotypic analyses of these strains is ongoing. In conclusion, *A. baumannii* encodes numerous, highly conserved putative sRNAs and available data suggests that at least some of these are true sRNA molecules that regulate small subsets of genes in AB307-0294.

**43**

## Regulatory RNA interactome of methicillin-resistant *Staphylococcus aureus* reveals genes required for antibiotic tolerance.

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*Staphylococcus aureus* is a major cause of skin, soft tissue, respiratory and endovascular infections. The emergence of antibiotic-resistant strains, including methicillin-resistant *S. aureus* (MRSA), has limited the treatment options to last-line antibiotics like vancomycin, and increased the burden on the health-care system. Populations of MRSA with intermediate levels of vancomycin resistance (VISA) have emerged and are commonly associated with vancomycin treatment failure. These strains are associated with distinct physiological changes including cell wall thickening and reduced autolysis. Transcriptional profiling of MRSA isolates following exposure to antibiotics indicate that regulatory small RNA (sRNA) expression is correlated with antibiotic stress, although the precise function of these gene regulators is unknown.

Here we have performed UV crosslinking, ligation and sequencing of hybrids (CLASH) to profile the *in vivo* sRNA-mRNA interaction network and identify functions for regulatory sRNAs in the MRSA isolate JKD6009. Multiple sRNA-mRNA interactions were verified experimentally, confirming the robustness of our approach. Using CRISPR interference (dCas9) to knock down expression of sRNAs interacting with cell wall synthesis or antibiotic tolerance-associated mRNAs, we have screened for vancomycin sensitivity phenotypes. One sRNA, here termed the vancomycin-intermediate small RNA (*visA*), exhibited severe vancomycin and teicoplanin sensitivity, suggesting a glycopeptide-specific sRNA response. Vancomycin sensitivity was recapitulated in a Δ*visA* mutant and vancomycin tolerance was restored in a Δ*visA*::*visA* chromosomal repair strain confirming that *visA* is required for vancomycin tolerance in VISA isolates. Additionally, the identified *visA* regulon includes both novel mRNAs and sRNA sponging interactions. One such mRNA interaction identified by CLASH was the lytic transglycosylase *isaA* involved in cell wall expansion and turnover. Knock down of *isaA* also rendered cells vancomycin-sensitive resembling the Δ*visA* strain. Finally, we show Δ*visA* has significantly decreased cell wall thickness when compared to wild-type, which most likely contributes to the increased vancomycin sensitivity seen. These results demonstrate a novel mechanism of antibiotic tolerance through sRNA *visA* regulation of *isaA* and cell wall turnover during antibiotic stress.

**44**

## Salmonella peptidoglycan biosynthesis inside eukaryotic cells: novel enzymes impacting antibiotic resistance

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Our recent studies support structural rearrangements in the peptidoglycan (PG) of *Salmonella* enterica serovar Typhimurium (S. Typhimurium) when this pathogen resides within acidic phagosomes of eukaryotic cells. These changes respond to an intricate regulatory network affecting several of the known PG enzymes as well as novel enzymes that this bacterium acquired during its evolution and are absent in non-pathogenic bacteria. The changes in PG structure occur concomitantly to the activation of virulence factors used by intracellular bacteria to survive inside the

phagosomal compartment. Some of the novel PG enzymes up-regulated by intracellular *S.* Typhimurium bind antibiotics with lower affinity compared to enzymes used by extracellular bacteria. These observations alert us of the necessity for developing new drugs targeting specifically PG biogenesis in the intracellular enviroment and the possibility of immune evasion mechanisms taking place inside the infected cell based on modifications in the PG built by intracellular bacteria.

**45**

## ARDaP: Antimicrobial Resistance Detection and Prediction from Whole-Genome Sequence Data

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Antibiotic resistance (AbR) is a major threat to human health worldwide[1]. Whole-genome sequencing (WGS) is rapidly changing the clinical microbiology landscape, with exciting potential for rapidly and accurately detecting AbR. Most work to date has focussed on developing software to detect gene presence[2]. Unfortunately, much less consideration has been given to identifying chromosomally-encoded AbR mechanisms.

We present software for Antibiotic Resistance Detection and Prediction (ARDaP) from WGS data. ARDaP was designed with two main aims: 1) to accurately identify all characterised AbR mechanisms and present the AbR profile in an easy-to-interpret report; and 2) to predict enigmatic mechanisms based on i) novel mutants in known genes, or ii) a microbial genome-wide association approach that correlates AbR phenotypes with genetic variants.

We demonstrate the application of ARDaP using *Burkholderia pseudomallei* as a model organism due to its exclusively chromosomally-encoded AbR mechanisms and high mortality rate[3]. Using a well-characterised collection of 1,042 clinical strains, we demonstrate that ARDaP accurately detects all known AbR mechanisms in *B. pseudomallei* (>40 mutations) with high rates of precision and recall. Furthermore, ARDaP predicted three novel loss-of-function mutations that decreased meropenem susceptibility in *B. pseudomallei*; this phenotype is associated with increased treatment failure and fatality rates[3].

ARDaP is a comprehensive and accurate tool for identifying and predicting AbR mechanisms from WGS data. Its clinician-friendly report[4], which summarises a given strain's AbR profile, holds great promise for informing personalised treatment regimens and treatment shifts in response to the detection of precursor or AbR-conferring mutations.

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2. 2) Jia B, Raphenya AR, Alcock B, et al. CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. Nucleic Acids Res, 45, 566-573 (2017).
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**46**

## The Emergence of Pandemic Fluoroquinolone-Resistant Uropathogenic *Escherichia coli* Clones in Australia

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**Introduction:** Increasing resistance to fluoroquinolone antibiotics amongst uropathogenic *Escherichia coli* is of critical concern to public health. Resistance is mainly driven by the sequence type (ST)131 C2/H30Rx sub-lineage, however national surveillance in Australia reports increasing cases of both fluoroquinolone-resistant ST131 and ST1193. Despite this, limited genomic investigations have been undertaken nation-wide.

**Methods:** Here, we analysed whole-genome sequence data of ST131 (*n*=183) and ST1193 (*n*=65) isolates collected across Australia between 2001-2014. Long-read sequencing of several isolates enabled the genomic context of genes encoding antimicrobial resistance and virulence to be determined. We contextualised our Australian dataset with well-characterised published genomes to investigate spatial clusters and lineage diversity.

**Results:** Most ST131 isolates were from clade C (*n*=193, 92.3%) and contained mutations in *gyrA* and *parC* conferring fluoroquinolone resistance ($FQ^R$). In comparison, $FQ^R$ in ST1193 is mediated by different mutations in the same chromosomal genes. Bayesian analysis predicted that fluoroquinolone-resistant ST131 and ST1193 emerged in 1987 and 1989 (respectively), coinciding with increased usage of fluoroquinolones worldwide. Acquisition of fitness and uropathogenicity genes likely primed ST131 for success before the development of $FQ^R$. Conversely, ST1193

is characterised by recombination of a 30.4 kb region encompassing the capsular biosynthesis genes causing a switch from the K5 to K1 capsular antigen, associated with altered host immune evasion. This capsule switch in an already $FQ^R$ background enhanced virulence and is associated with expansion of the major ST1193 sub-lineage. In comparison, six group two capsular polysaccharides are present across ST131, with the K5 capsular antigen the most prevalent (*n*=96, 45.9%). ST1193 and ST131 share a genomic island inserted at tRNA-*asnT* carrying the same siderophore-dependent iron uptake systems and putative adhesins.

**Conclusion:** ST131 and ST1193 have disseminated across Australia following the independent acquisition of mutations in genes conferring $FQ^R$. ST131 underwent massive population expansion following the acquisition of $FQ^R$ in 1987, while recombination in the capsular region appears to have driven the expansion of $FQ^R$ ST1193.

**47**

## Contribution of plasmid-mediated ciprofloxacin resistance to the fitness of *Escherichia coli* ST131

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The uropathogenic *Escherichia coli* (UPEC) ST131 clone is of significant clinical importance due to its multidrug resistance phenotype and high prevalence worldwide. Genomic analyses have classified ST131 into three major clades (A, B and C), with all strains from clade C containing mutations in *gyrA* (L83S, N87D) and *parC* (I80S) that together confer high-level resistance to fluoroquinolones (FQs). Clade C strains can be further subdivided into clade C1 and C2. The plasmid encoded enzyme AAC(6')-Ib-cr confers reduced susceptibility to ciprofloxacin (CIP) via acetylation of its piperazinyl group, and may prime the development of high-level resistance via mutations in *gyrA* and *parC*. Curiously, the presence of *aac(6')-Ib-cr* gene is associated with clade C2 but not C1 strains (70.7% vs 3.7%), suggesting that *gyrA* and *parC* mutations were developed before the acquisition of *aac(6')-Ib-cr* gene in clade C2. Here we sought to understand the role of the *aac(6')-Ib-cr* gene in clade C2 strains highly resistant to FQs. We adapted a method utilizing transient suppression of DNA repair to perform gene editing on the chromosome of the reference clade C2 strain EC958 and reverted the *gyrA* and *parC* mutations (alone and in combination) back to *E. coli* K-12 FQ-sensitive. Using these revertants, we showed that AAC(6')-Ib-cr conferred a 2-4 fold increase in MIC to CIP in all strain backgrounds. An EC958 mutant lacking *aac(6')-Ib-cr* can be out-competed by EC958wt in human urine supplemented with CIP (64 mg/L). Furthermore, EC958 carrying the *aac(6')-Ib-cr* gene is capable of reducing the concentration of CIP in the media by converting CIP to its inactive form acetylated-CIP. Thus, the *aac(6')-Ib-cr* gene may play an active role in the survival of C2 strains in urine of patients undergoing FQ treatment. This finding challenges the belief that the *aac(6')-Ib-cr* gene is only important in FQ-sensitive strains, and suggests its acquisition contributes to the fitness of ST131 multidrug resistant clade C2 strains.

**48**

## Mobilization of Antimicrobial-Resistance Plasmids in *Staphylococcus aureus*

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*Staphylococcus aureus* is a common cause of hospital, community and livestock-associated infections and is increasingly resistant to multiple antimicrobials. In *S. aureus* the majority of antimicrobial-resistance and virulence genes are carried on extrachromosomal plasmids. Only around 5% of sequenced *S. aureus* plasmids are considered self-transmissible conjugative plasmids, however 92% of non-conjugative plasmids are predicted to be mobilisable. The pWBG749 family of staphylococcal conjugative plasmids mobilize non-conjugative plasmids carrying mimics of the pWBG749 origin-of-transfer (*oriT*) sequence. These *oriT* mimics are carried by 53% of non-conjugative *Staphylococcus aureus* plasmids. The *oriT*s have diverged into five subtypes. Variants of the ribbon-helix-helix-domain protein SmpO, encoded by each conjugative plasmid, determine specificity for each *oriT*. Moreover, conjugative plasmids can mobilize non-cognate *oriT*s if the appropriate SmpO variant is present. Here we characterized four SmpO variants using surface-plasmon-resonance (SPR)-based DNA-binding assays, analytical gel filtration, small-angle X-ray scattering and other techniques. The SmpO proteins formed tetramers in solution and bound two DNA sites, IR2 and IR2*, located ~60 bp apart. SPR using single-nucleotide-substituted oligonucleotides delineated the critical nucleotides for three different SmpO-IR2* interactions. Each SmpO protein specifically bound a distinct IR2/IR2* motif, however, a single amino-acid substitution in SmpO enabled a switch in SmpO binding and *oriT*-mobilization specificity. Interestingly, two divergent conjugative plasmids, pWBG731 and pCO2, mobilized the same group of *oriT* sequences. This and other phylogenetic incongruences between conjugative plasmids and their *oriT* sequences were observed in multiple pWBG749-family lineages. Therefore, not only have conjugative plasmids diverged to carry distinct *oriT* sequences, but strangely, conjugative-plasmid *oriT* sequences have also seemingly converged—or have been replaced—with pre-existing *oriT* types on non-conjugative plasmids during evolution. We propose this apparent '*oriT* switching' results from strong selection for mobilization of non-conjugative plasmids carrying an incompatible *oriT*. These observations suggest *oriT* specificity changes frequently during evolution and relaxase-*in trans* conjugative mobilization mechanism is likely a driving factor.

**49**

## Understanding uropathogenic *E. coli* resistance and virulence.

**Mark Schembri**[1]

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Uropathogenic *Escherichia coli* (UPEC) are a major cause of urinary tract and bloodstream infections, and a significant driver of increasing antibiotic resistance. Several globally dominant UPEC lineages have been described, the most common being the multidrug resistant UPEC sequence type 131 (ST131) clone. ST131 was identified in 2008 as a major clone linked to the spread of the CTX-M-15 extended-spectrum β-lactamase (ESBL)-resistance. Since then, ST131 has also been strongly associated with fluoroquinolone resistance. In this presentation, I will discuss our recent work on the molecular characterisation of UPEC ST131, including the use of genome sequencing to demonstrate its rapid and recent global dispersal, and the development of a high-throughput transposon mutagenesis system in combination with next generation sequencing to understand the regulation of key virulence factors and the spread of antibiotic resistance genes.

**50**

## Need-based activation of antibiotic resistance

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Resistance against antimicrobial peptides in many Gram-positive bacteria is mediated by detoxification systems that are comprised of a two-component regulatory system and an ATP-binding-cassette (ABC) transporter. The histidine kinases of these systems depend entirely on the transporter for detection of antimicrobial peptides, suggesting a novel mode of signal transduction where the transporter constitutes the actual sensor. We have taken a multi-disciplinary approach combining experimental and computational methodology to unravel the mechanistic details of this unique signaling pathway, exemplified by the bacitracin resistance system BceRS-BceAB of *Bacillus subtilis*. We show that the transporters and kinases form a sensory complex in the membrane, and that the histidine kinase directly responds to changes in the activity of the transporter. This appears to occur via complete control of kinase conformation by the transporter, which to our knowledge is unprecedented among bacterial signaling systems. We propose that this novel regulatory strategy effectively implements a flux-sensing mechanism, allowing the cell to adjust the rate of *de novo* transporter synthesis to precisely the level needed for protection.

**51**

## Antimicrobial fatty acids impact membrane biology and antibiotic resistance in *Acinetobacter baumannii*

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Free fatty acids hold important immune-modulatory roles during infection. However, the host's long chain polyunsaturated fatty acids, not commonly found in the membranes of bacterial pathogens, also have significant broad-spectrum antibacterial potential. Of these, the omega-6 fatty acid arachidonic acid (AA) and the omega-3 fatty acid decosahexaenoic acid (DHA) are highly abundant, hence, we investigated their effects on *Acinetobacter baumannii*. Our analyses reveal that AA and DHA readily incorporate into the *A. baumannii* membrane and impact membrane integrity. Importantly, our analyses also reveal a role for environmental fatty acids in antibiotic susceptibly and the development of antibiotic resistance in *A. baumannii*. Through transcriptional profiling and mutant analyses, we identified multiple lipid homeostasis mechanisms that play a role in AA and DHA resistance, including the b-oxidation pathway, the AdeIJK RND efflux system and a DesB-like desaturase. This is the first study to examine the antimicrobial effects of host fatty acids on *A. baumannii*, and highlights the potential of AA and DHA to protect against *A. baumannii* infections. Further, the novel role for fatty acids in antimicrobial resistance provides insights into the complex interplay between host factors and anti-*A. baumannii* therapy.

**52**

## Overcoming antibiotic resistance: Inhibiting the AmpC β-Lactamase Induction Pathway

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The therapeutic use of β-lactam antibiotics is being steadily eroded by the increasing prevalence of resistance mechanisms, and since β-lactams are the front line of defense against infection this development is of great concern. A newly emerging threat are the AmpC class of β-lactamases. Genes encoding AmpC are found in many Gram-negative bacteria including several opportunistic pathogens that show resistance to numerous antibiotics and cause life-threatening infections in immunocompromised patients. Due to the spread of AmpC and the fact that this enzyme is resistant to many clinically available β-lactamase inhibitors, new strategies for coping with this resistance mechanism are of current interest.

The production of AmpC β-lactamase within many of these organisms depends critically on the activity of an enzyme, known as NagZ, which acts to generate a peptidoglycan catabolite that then interacts with the transcriptional regulator of *ampC*. Under normal conditions the *ampC* gene is not transcribed however, when β-lactams are present the cellular concentration of this NagZ produced catabolite increases and AmpC production is stimulated.

Inhibitors of NagZ should block accumulation of the key peptidoglycan catabolite, thereby blocking the production of AmpC and rendering these bacteria once again susceptible to β-lactams. Here the rationale and evaluation of compounds as inhibitors of NagZ is discussed. Several potent and selective compounds are presented and a structural rationale for inhibitor potency and selectivity is also described. Furthermore, these inhibitors reduce the MIC values for several clinically relevant cephalosporins in both wild-type and AmpC hyper-producing bacterial strains in the presence of clinically relevant β-lactams. These compounds should prove useful in overcoming AmpC-mediated resistance in Gram-negative bacteria, and could lead to an effective treatment of otherwise dangerous antibiotic resistant bacterial infections.

**53**

## Sulfonamide inhibitors of carbonic anhydrases impact multiple targets

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The carbonic anhydrase inhibitors acetazolamide (AAZ) and methazolamide (MZA) are safe, clinically used drugs originally designed to treat non-bacteria related illnesses (*e.g.* glaucoma). But they also show antimicrobial activity against the gastric pathogen *Helicobacter pylori.* AAZ in particular is sufficiently effective to promote ulcer healing and to prevent ulcer recurrence, indicative of its potential as an anti-*H. pylori* drug. In this study, the inhibitory activities of AAZ, MZA and ethoxzolamide (EZA) were measured against several *H. pylori* laboratory and clinical strains. Mutants resistant to these compounds were isolated, characterized, and their genomes sequenced. The results show that AAZ, EZA and MZA kill *H. pylori* via mechanisms that are different from the mode of action of common antibiotics used to treat *H. pylori* infections, as they retain activity against antibiotic-resistant clinical isolates. Acquisition of resistance to sulfonamides in the laboratory was associated with a complex phenotype and genetic mutations in up to 12 genes, including the one encoding undecaprenol pyrophosphate synthase, a known target of sulfonamides. The data suggest that sulfonamides impact multiple targets in killing *H. pylori*. The frequency of single-step spontaneous resistance acquisition by *H. pylori* was low, showing that resistance does not develop easily. The antimicrobial activity of these compounds is restricted to *H. pylori* and a few other bacteria, including *Streptococcus pneumoniae*, *Neisseria* spp. and *Brucella* suis. Our findings suggest that this class of approved drugs can be developed into selective anti-*H. pylori* agents with a novel mechanism of action.

**54**

## A novel mechanism for translational regulation of linezolid resistance in *Staphylococcus aureus*

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**Abstract**

Linezolid has become a last defence drug for the treatment of methicillin-resistant *Staphylococcus aureus.* The *cfr* gene confers resistance to multiple phenicol antibiotics and linezolid through methylation of the 23s rRNA[1,3] and is often carried on multidrug-resistance plasmids. Recently, a new chromosomally-integrated variant of the *cfr* gene was discovered in pig and human-isolated ST398 *S. aureus* in Australia[2]. This allele, dubbed *cfrAB,* contains a single base-pair deletion resulting in a premature stop codon in the first half of the ORF. Despite this, the *cfrAB* allele provides low-level linezolid and phenicol resistance, suggesting full-length Cfr methyltransferase is produced. Here we show that the *cfrAB* allele contains a programmed ribosomal frameshift site capable of inducing translational slippage to the -1 frame, facilitating full translation of Cfr. Fusion of the frameshift site between maltose-binding protein and GFP genes facilitated detection of the frameshifted protein product by SDS-PAGE and mass spectrometry. Fusion of the frameshift site to *lacZ* allowed quantitative detection of frameshifting through β-galactosidase assays and indicated that in *E. coli* this site produces around 2% the level of translation compared to the in-frame allele. Preliminary experiments also suggest sub-inhibitory levels of antibiotics such as florfenicol may stimulate frameshifting. Interestingly, the only human-isolated clone of this lineage carries the full-length *cfr*, suggesting a reversion of *cfrAB* to *cfr* may have occurred *in situ*. Consistent with this, *crfAB* to *cfr* mutations were detected at a rate of $1 \times 10^{-8}$ in stationary-phase cells. We suspect that the *crfAB* allele may represent a recently evolutionary adaptation to facilitate reduced and potentially regulated resistance, which may confer a competitive fitness benefit. This chromosomally-integrated *cfrAB* allele may therefore facilitate persistence of this resistance mechanism in ST398 populations – and also may go undetected in routine phenotypic screens for resistance.

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**55**

## Polymyxin resistance: Deciphering the interplay of different lipid A modifications in *Klebsiella pneumoniae*

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Polymyxins are the last resort against multidrug-resistant Gram-negative bacteria; however, polymyxin resistance is emerging, including in a major nosocomial pathogen *Klebsiella pneumoniae*. The common resistance mechanisms involve lipid A modifications with phosphoethanolamine(pEtN) and 4-amino-4-deoxy-L-arabinose(L-Ara4N) mediated by chromosomal *eptA* and *arnT*, respectively. The recent discovery of plasmid-encoded pEtN transferase gene, *mcr*, indicates potential rapid horizontal dissemination of polymyxin resistance. This study aimed to investigate the interplay between pEtN and L-Ara4N transferase genes, *mcr-1*, *eptA* and *arnT*, in conferring polymyxin resistance in *K. pneumoniae*. Clinical *K. pneumoniae* II-503 carrying *mcr-1* on a native plasmid was used to construct markerless deletion mutants. Single, double and triple deletions of *mcr-1*, *eptA* and *arnT* mutants were generated using the FLP/FRT recombination system. Polymyxin susceptibility was determined using MICs and static time-kill. Lipid A profiling was examined using LC-MS. All the *mcr-1*-carrying strains were resistant to polymyxin B with MICs of 8 mg/L, and static time-kills with polymyxin B at 8 mg/L were ineffective, indicating that the *mcr-1* was the predominant polymyxin resistance mechanism. Reduced MICs of 0.5mg/L was observed for mutants with *mcr-1* deleted. The static time-kills revealed significant killing of all *mcr-1*-deletion mutants with >3-$\log_{10}$cfu/mL reduction in bacterial viability at 1h following treatment. However, by 16h all these mutants demonstrated regrowth to the level similar to the control (~9 $\log_{10}$cfu/mL), with the exception of II-503Δ*mcr-1*Δ*eptA*Δ*arnT* which regrew to only 5.7±0.7 $\log_{10}$cfu/mL. Following regrowth, L-Ara4N-modified lipid A species were present in mutants that retained *arnT* but lacked *mcr-1* (II-503Δ*mcr-1* and II-503Δ*mcr-1*Δ*eptA*), while no modified lipid A species were observed in II-503Δ*mcr-1*Δ*arnT* and II-503Δ*mcr-1*Δ*eptA*Δ*arnT*. In conclusion, *mcr-1* and *arnT*, but not *eptA*, have major roles in conferring resistance towards polymyxin in *K. pneumoniae* II-503. The regrowth of the mutants lacking of both *mcr-1* and *arnT* could due to other resistance mechanisms, which is currently under investigation using lipidomics and transcriptomics.

**56**

## A regulatory small RNA is embedded within the Shiga toxin transcript

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Enterohaemorrhagic *E. coli* (EHEC) is a significant human pathogen that causes disease ranging from acute diarrhoea to potentially life threatening haemolytic uremic syndrome (HUS) and renal failure. The most severe disease symptoms are caused by the release of Shiga toxins, AB5-family toxins that depurinate 28S ribosomal RNA in Gb3-rich renal endothelium cells. Shiga toxins are encoded within the late transcript of lambdoid bacteriophages and are transcribed from the late promoter, $P_{R'}$. The late promoter is constitutively active, but constitutively terminated at $t_{R'}$ downstream of the late promoter during lysogeny. Transcription and release of the Shiga toxin is regulated by lytic induction of the phage and anti-termination of the late transcript. We have found that the short $t_{R'}$ terminated transcript is bound by the small RNA chaperone Hfq and accumulates to high levels in StxΦ lysogens. The $P_{R'}$ transcript, here termed Stx small RNA (StxS), is processed into a shorter 74nt sRNA by the major endoribonuclease RNase E. Surprisingly, the StxS sRNA is not part of the StxΦ regulatory network and phage propagation is not affected in a Δ*stxS* mutant. We find that StxS-mRNA interactions were recovered in our recent sRNA-mRNA interaction network analysis (using RNase E-CLASH) and that StxS interacts with the stationary phase stress response sigma factor, RpoS. Using GFP transcriptional and translational fusions we demonstrate that StxS activates RpoS translation 5-fold, and that StxS is required for wild-type induction of RpoS in EHEC. StxS activation of *rpoS* mRNA is not required for osmotic or acid tolerance, but promotes high cell density growth under nutrient-limited conditions. These results suggest that StxS may allow host colonisation at higher cell densities, potentially leading to higher Shiga toxin titres and an increased probability of developing severe disease symptoms including HUS.

**57**

## Anti-sporulation strategies targeting *Clostridioides difficile* and other spore-forming bacterial pathogens

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Spore-forming bacteria include the devastating human pathogen *Clostridioides difficile,* the food spoilage pathogen *Bacillus cereus* and the bioterrorism agent *Bacillus anthracis*. Spores are the infectious particles of these pathogens and their resistant structure makes their eradication difficult. Their persistence properties enable the spread of disease, resulting in fatalities and economic devastation in health care settings, the food industry and public spaces in the case of weaponised anthrax. Despite this major burden, there are no strategies preventing spore production.

*C. difficile* is of considerable medical interest due to the disease burden and global challenge of managing the consequences of infection. Spores are a crucial mediator of *C. difficile* disease initiation, dissemination and re-infection and are highly resistant to current therapeutics.

Here we report the novel finding that cephamycin antibiotics can directly impact *C. difficile* spore production by inactivating spore-specific proteins. Through TEM imaging we observed that cephamycins block early sporulation stages. We found that this sporulation inhibition phenotype is generic

using the spore-forming pathogens *Paeniclostridium sordellii* and *B. cereus*, a close relative of *B. anthracis*, confirming that cephamycins have broad applicability in anti-sporulation strategies.

The major anti-sporulation molecular target of the cephamycins was identified as a spore-specific penicillin-binding protein, SpoVD. Sporulation assays using a *spoVD* mutant confirmed its essential role in sporulation. SpoVD is found in other spore-forming bacteria, including *B. anthracis* and *B. cereus*. Through our binding assays we showed that the cephamycins target SpoVD in these pathogens.

Of clinical relevance, we found that co-treatment of mice with the cephamycin, cefotetan and the current primary *C. difficile* treatment vancomycin prevented disease re-infection. Our findings could therefore directly and immediately impact *C. difficile* infection treatment. Importantly, our results also provide new insights into disease prevention through targeting of the sporulation process, for the first time for any spore-forming pathogen. They also provide evidence of potential new anti-sporulation targets that could significantly advance drug development for other important spore-forming pathogens.

**58**

## Peptidyl-prolyl isomerase, *ppiB*, is essential for proteome homeostasis and virulence in *Burkholderia pseudomallei*

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*Burkholderia pseudomallei* is the causative agent of melioidosis, a disease endemic in South-East Asia and northern Australia. Mortality rates in these areas are high even with antimicrobial treatment, due to few options for effective therapy. Therefore there is a requirement to identify anti-bacterial targets for the development of novel, effective treatments. Cyclophilins are a family of highly conserved enzymes shown to be important in multiple cellular processes. Cyclophilins catalyse the *cis-trans* isomerization of *xaa*-proline bonds, a rate limiting step in protein folding which has been shown to play a role in bacterial virulence. *B. pseudomallei* strain K96243 encodes for a cytoplasmic cyclophilin B gene, *ppiB*, the role of which was investigated in this study. A cyclophilin B-null mutant strain, *BpsΔppiB,* was generated and characterised. *In vitro* macrophage invasion studies showed that *BpsΔppiB* had 36.8-fold reduction in intracellular numbers at 9 hours, which upon further investigation using immunofluorescence showed that there was a decrease in cell-to-cell spread and fusion of macrophages into Multi-nucleated Giant Cells (MNGC). *BpsΔppiB* is avirulent in the BALB/c mouse infection model with all survivors clearing infection. *BpsΔppiB* also displayed a reduction in motility and biofilm formation as well as an increase in susceptibility to oxidative stress and antibiotics. To determine the mechanisms behind the reduction in virulence, global proteomic analysis was conducted and demonstrated the loss of PpiB leads to widespread disruption of the proteome with 734 proteins undergoing statistically significant alterations. The loss of PpiB leads to decreased abundance of multiple virulence determinants including flagellar machinery, chemotaxis detection systems, capsular polysaccharide and alterations in Type-VI secretion systems-associated proteins. The alteration in these protein levels supports our hypothesis that PpiB is important in maintaining proteome homeostasis and thus virulence in *B. pseudomallei*.

**59**

## Protein longevity as a wake-up call for dormant cells

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All living organisms require nutrients to grow and reproduce. When nutrient quantity or quality is low, organisms reduce their growth rate and enter a dormant state characterized by arrested physiological activity and critical for cell survival. We now report that preserving proteins during dormancy speeds the return to a growth state. We establish that the bacterium Salmonella enterica reduces proteolysis by adenosine triphosphate (ATP)-dependent proteases by decreasing ATP amounts when starved for magnesium, carbon or nitrogen. The yeast Saccharomyces cerevisiae also reduces ATP amounts and ATP-dependent proteolysis when starved for nutrients. Drugs that increase ATP amounts delay entry into the growth state by promoting ATP-dependent proteolysis. Thus, the better the ability to preserve proteins during dormancy, the faster prokaryotes and eukaryotes exit the dormant state as soon as nutrients become available. Starvation-promoted protein longevity likely also plays a role in the germination of bacterial spores and in antibiotic persistence.

**60**

## Biological insights into the mechanisms that define the lipidomic landscape of *Acinetobacter baumannii*

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*Acinetobacter baumannii* is a Gram-negative nosocomial pathogen associated with significant morbidity and mortality in susceptible individuals. A number of persistence and resistance strategies contribute to the success of this organism, including an ability to alter the biophysical properties of the membrane in response to changing environmental conditions. This process is achieved, in part, by the fatty acid and phospholipid biosynthetic pathways. However, the molecular basis and the interplay of these lipid homeostasis mechanisms in *A. baumannii* is largely ill-defined. We have identified critical roles of two related, but functionally distinct desaturases in unsaturated fatty acid production and defined that these are co-ordinately regulated by local fatty acid sensing regulators. To understand the relative contribution of the desaturases in defining the *A. baumannii* lipidome, individual deletion derivatives were examined for their ability to persist across a range of physiologically relevant growth conditions, including osmotic pressure and nutrient depletion. The fatty acid composition and the subsequent biophysical properties of the membrane were also determined. Given the essentiality of fatty acid and phospholipid biosynthesis in bacterial viability, and their critical role in stress adaptation, investigations into the mechanisms of lipid homeostasis are crucial for understanding how this pathogen of global significance adapts to host-induced and environmental stress.

**101**

## Multidrug resistance analysis of community acquired urinary tract infections in the coastal region of Bangladesh

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Urinary tract infection (UTI) is one of the most common causes of bacterial infection in the rural part of Bangladesh. Bacterial prevalence and their antibiotic resistance patterns in rural coastal region might be different due to high salt concentration in household water. This study demonstrates the prevalence of urinary pathogens and their antibiotic resistances isolated from community individuals in two coastal areas (Shyamnagar-22°20'14.2764''N and 89°6'31.1400''E ; Paikgachha-22°35'20.04"N and 89°20'9.96"E) of Bangladesh. In this study, urine samples were collected from 116 individuals using mid-stream clean-catch method. Bacterial identification and antibiotic resistance were observed using standard laboratory procedure. The prevalence of UTI was 9% (5 out of 55) in males and 20% (12 of 61) in females, although asymptomatic bacteriuria was 93% (108 of 116) in all studied individuals. Among the isolates, 69% (n=80) were Escherichia coliof which 69% (55 of 80) were multidrug resistant (MDR). Staphylococcus saprophyticus was present in 50% (n=58) and Enterobacter aerogenesin 28% (n=32) individuals of which 60% (18 of 30) and 91% (3 of 32) had MDR strains respectively. The prevalence of other bacteria were as follows: 21% (n=24) P. aeruginosa,17%(n=20) S. aureus, 11% (n=13) K. pneumonia and Staphylococcus spp. each, 7% (n=8) Citrobacter sp., and 2% (n=2) Salmonella sp.The MDR rates were 83% (20 of 24) in P. aeruginosa, 80% (16 of 20) in S. aureus, 85% (11 of 13) in K. pneumonia and 60% (3 of 5) in Staphylococcus spp. Furthermore, the resistance rates against fluoroquinolone and third generation cephalosporin were also abundant; 52% and 40% in Escherichia coli, 43% and 23% in Staphylococcus saprophyticus, 38% and 44% in Enterobacter aerogenes, 31% and 23% in K. pneumonia respectively. These results suggest that resistance to common antibacterial drugs is already quite significant in the study population and most likely will grow with time, which warrants area-specific monitoring data for choosing efficacious and empirical treatment for UTIs. Healthcare practitioners should also be aware of potential treatment failure.

**102**

## Differential role of the K1 and K5 capsule in virulence of the fluoroquinolone-resistant uropathogenic *Escherichia coli* ST1193 clone

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Uropathogenic *Escherichia coli* (UPEC) is the most common cause of urinary tract infections (UTIs). The increase in the human use of fluoroquinolones as a first-line agent for uncomplicated UTIs has contributed to the emergence and spread of multidrug-resistant strains. Indeed, our group has studied the genomic epidemiology and virulence of sequence type (ST)131, the most globally dominant fluoroquinolone-resistant UPEC clone and a major cause of UTI and bloodstream infections associated with limited treatment options. Recent surveillance has identified ST1193 as the second most dominant fluoroquinolone-resistant UPEC clone (after ST131). The global emergence of ST1193 follows a similar timeline to ST131, and sequence analysis has revealed the ST1193 linage consists of two major clades. These two clades are distinguished by recombination of a 30.4 kb region encompassing the capsular biosynthesis locus, causing a switch from K5 (clade 2) to K1 (clade 1). To study the involvement of the capsule in the virulence of the ST1193 linage, two K1 and two K5 capsule representative strains were studied. Mutation of the *kpsD* gene, which encodes an outer membrane protein essential for surface expression of the capsule polysaccharide, abolished capsule production in all strains as demonstrated using lytic K1 and K5 specific phage. Survival of the *kpsD* mutants in whole blood was also examined using single and mixed competitive assays. While the wild-type strains were able to survive in whole blood, survival of the *kpsD* mutants was highly

attenuated. We have now tagged the K1 and K5 wild-type strains using different antibiotic resistance gene markers, and are examining the differential contribution of the polysialic acid-containing K1 capsule compared to the glycosaminoglycan-containing K5 capsule in ST1193 virulence.

**103**

## Multiple bacterial veterinary pathogens contain phase-variable regulons; phasevarions.

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Many bacterial pathogens contain randomly-switching methyltransferases that control phase-variable regulons – phasevarions. All current examples control expression of genes involved in pathogenesis and host-adaptation, and many regulate putative and current vaccine candidates. Effective vaccines require stably expressed targets; individual phase-variable genes can be identified *in silico* as they contain easily recognised features, but genes controlled by phasevarions do not, complicating the rational design of vaccines. We have identified and begun to study phasevarions controlled by the switching of both Type I and Type III methyltransferases in several important bacterial veterinary pathogens: *Streptococcus suis* and *Actinobacillus pleuropneumoniae* are major swine pathogens, with *S. suis* also a major cause of bacterial meningitis in humans, particularly in S.E. Asia; *Mannheimia haemolytica* is a major bovine pathogen; multiple species of the *Mycoplasmataceae*, obligate intracellular pathogens, cause disease in a range of livestock. All these organisms contain both Type I and Type III methyltransferases that are able to phase-vary, a phenomenon never before observed in individual species. Pacific Biosciences SMRT sequencing and methylome analysis of the methyltransferases from these organisms has deciphered their specificity, and demonstrated that different genes/alleles methylate different target sequences, therefore controlling different phasevarions. Analysis of the protein profiles of strains containing phase-variable methyltransferases shows protein expression differences correlating with methyltransferase switching.

Our analysis shows that phasevarions are present in diverse veterinary pathogens, and need to be characterised in order to identify the stably expressed protein repertoire of these organisms. This work will provide a framework for the rational design of vaccines and treatments against these bacteria

**104**

## Genetic basis of Group A *Streptococcus* cotrimoxazole resistance

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Impetigo is a skin infection caused by Group A *Streptococcus* (GAS) that is highly prevalent in Indigenous Australian Aboriginal and Torres Strait Islander communities. Approximately 45% of Indigenous children living in northern Australia are affected by impetigo at any given time, which is the highest prevalence in the world. While GAS is the key impetigo pathogen, co-infection with *S. aureus* is common, and both pathogens can cause a number of severe complications that include Streptococcal and Staphylococcal sepsis, and immune sequelae of GAS infection. In an effort to reduce the burden of impetigo in endemic populations, the antibiotic combination cotrimoxazole (SXT) has shown considerable promise. However, with expanding use there is concern that resistance could emerge. Resistance to SXT requires resistance to each of the component antibiotics: sulfamethoxazole (SX) and trimethoprim (TP). While the requirement for resistance to both TP and SX reduces the likelihood of SXT resistance occurring, there is concern that genes or mutations that confer resistance to TP and/or SX might disseminate within Gram-positive skin pathogens while remaining undetected by routine clinical microbiology surveillance. In particular, the TP-resistance gene *dfrG* is already circulating in ~10% of methicillin-resistant *S. aureus* (MRSA) isolates in northern Australia. In this study, through a combination of phenotypic testing, *in vitro* selection and genomic analysis, we show that *dfrG* does not confer SXT resistance, even for GAS strains that have also acquired high-level SX resistance. This important finding alleviates the concern that *dfrG* has the potential to lead to SXT-resistant GAS (and potentially MRSA) infections in Northern Australia, and suggests that the potential for GAS to become to SXT as a result of horizontal gene transfer from MRSA coinfections is low.

**105**

## Investigating the Tat translocation of β-lactamases in Gram negative bacteria

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β-lactamases are the most common agents of antimicrobial resistance in bacteria (Bush *et al.,* 1995). These enzymes translocate to the periplasm via the Sec or Tat pathway (Pradel *et al.,* 2008) depending on their signal sequence at the N terminal (Berks *et al.,* 2003). Most well-known β-lactamases are known to translocate via the Sec pathway, which transports unfolded proteins across the inner membrane while a few are known to proceed via the Tat pathway which transports folded proteins. In this project, we aimed to investigate the basis of this differential β-lactamase translocation by studying the translocation of BKC-1, a plasmid-borne carbapenemase recently reported in Brazilian clinical strains of *Klebsiella pneumoniae* (Nicoletti *et al.,* 2015).

Signal peptides of various β-lactamases from BLDB were analysed using TatP and sequence alignment was carried out using Clustal Omega. Antibiotic sensitivity assays were carried out using broth dilution and protein expression profiles in whole cell lysates and periplasmic fractions were determined using Western blots.

Among the 1089 β-lactamase sequences that were fed into TatP, only 14, including BKC-1, were predicted to be Tat translocated. BKC-1 from *K.pneumoniae* revealed to possess a repeating h region in the signal peptide when aligned with BKC-1 from *Shinella zoogloeoides*. Strains lacking TatC and expressing BKC-1 continued to depict beta-lactam resistant phenotypes similar to those of the wild type with the native signal sequence but yielded relatively sensitive phenotypes when this sequence was modified with a shorter hydrophobic chain. This was consistent with BKC-1 protein expression levels.

We thus suggest that translocation of BKC-1 is not only dependant on the presence of the twin arginine motif, but also on the hydrophobicity and length of the signal peptide and the type of organism. This study also suggests that a β-lactamase that may be originally Tat translocated can potentially modify its signal sequence or be dual-targeting when acquired by another organism depending on the similarity of the Tat complex between the organisms.

**106**

## Structure and function of the staphylococcal multidrug efflux pump QacA: the importance of helix 12

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*Staphylococcus aureus* is a major bacterial pathogen responsible for multidrug resistant hospital- or community-acquired infections with significant morbidity and mortality throughout the world. Alarmingly, this 'Superbug' has developed resistance to quaternary ammonium compounds (QACs) which are widely used as disinfectants and antiseptics in medical, industrial and household settings. A main underlying factor in this resistance is the expression of drug efflux pumps such as QacA, which is by far the most prevalent plasmid-encoded multidrug efflux pump found in clinical *S. aureus* isolates. QacA is able to confer resistance to >30 structurally different monovalent and bivalent cationic antimicrobial agents. To date, QacA structure-function relationships have not been fully resolved. QacA is comprised of 14 transmembrane segments (TMS) and TMS 12 has been proposed to be a component of the bivalent cation-binding region. To delineate the functional importance of TMS 12, 30 amino acid residues within putative TMS 12 and its flanking region were individually substituted with cysteine and the impact of these substitutions on QacA-mediated resistance and efflux activities assessed. Western blotting analyses showed all QacA mutants were expressed at levels similar to wild-type. Resistance profiling identified three residues in the target region that when mutated produce a decreased resistance capacity to at least one of the six representative QacA substrates, indicating the importance of these residues in interaction with specific substrates. Fluorimetric transport assays found two residues with impaired ethidium efflux activity suggesting their involvement in QacA substrate translocation process. Our results confirm the functional interplay between TMS 12 of QacA and bivalent cationic substrates. Further binding studies are underway to determine as to whether functionally important residues in QacA directly involve in substrate binding process. The emerging picture of detailed structure and function of QacA is an imperative step towards the ultimate goal of translating the findings into development of novel antimicrobials/inhibitors for countering QacA-mediated resistance.

**107**

## Transposon Directed Insertion Sequencing (TraDIS) to investigate genes required for survival during antimicrobial synergy in multidrug-resistant *Klebsiella pneumoniae*

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*Klebsiella pneumoniae* is a deadly, nosocomial pathogen causing severe respiratory infections and bacteraemia. The WHO has flagged multidrug-resistant (MDR) *K. pneumoniae* strains as an "urgent threat to human health". Resistance to all classes of antimicrobials is on the rise, meaning that infections are becoming increasingly difficult to treat. Thus, combination therapy is becoming an attractive last-line option.

Here, we use Transposon Directed Insertion Sequencing (TraDIS) to assay the contribution of each chromosomal gene in a MDR clinical isolate of the *K. pneumoniae* epidemic clone ST258 to its survival during antibiotic exposure. A dense TraDIS library of over 300,000 unique Tn*5* mutants was exposed to two passages in Mueller-Hinton broth (MHB) containing ciprofloxacin (cip), amikacin (amk) or imipenem (imp), either alone or in combination (cip+amk or cip+imp). Each drug was added to a medium at a sub-inhibitory concentration of antibiotic, and MHB without antimicrobial was used as a control. Sequencing was performed on a HiSeq2500 Illumina platform, and reads were mapped back onto the PacBio-sequenced reference genome, comparing insertion numbers for each gene in the treated cultures and the untreated control.

We examined genes that are only important for survival during exposure to antimicrobial combinations. A number of genes displayed a significant reduction in insertion mutants, only when the strain was exposed to both antimicrobial combinations, indicating "synergy-specific resistance". We confirmed, using single gene knock outs, that one representative gene (*ihfB*) was involved in resistance only during combinational treatment. We also identified genes involved in sensitivity to these combinations.

This study pushes forward the emerging field of antibiotic combination therapy by providing insights into the survival strategies of MDR *K. pneumoniae* during exposure to specific antimicrobial combinations. The information generated by the study opens future avenues to finding novel antimicrobial helper drug targets able to enhance the activity of known antimicrobial combinations against this important nosocomial pathogen.

**108**

## *Disseminated Tuberculosis (TB); A Potential Role for the Antigen 85 Complex.*

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Publish consent withheld

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**109**

## EngCP, an endo α-*N*-acetylgalactosaminidase, is involved in the virulence of *Clostridium perfringens*

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*Clostridium perfringens* is the causative agent of human clostridial myonecrosis and food poisoning. It is able to produce more than 20 different toxins, of which a-toxin and perfringolysin O have been shown to be involved in myonecrotic disease. Our previous work has shown that the RevSR two-component regulatory system is involved in regulating virulence in our mouse myonecrosis model. Microarray and RNAseq analysis of a *revR* mutant showed that expression of the genes encoding a-toxin and perfringolysin O were not significantly altered, implying that other factors may also play a role in virulence. The RNAseq data showed that the expression of the *engCP* gene (previously *CPE0693*) was significantly down-regulated in a *revR* mutant. A previous study had shown that EngCP had endo a-*N*-acetylgalactosaminidase activity and therefore could hydrolyse *O*-glycans. Similar enzymes from several Gram-positive pathogens have been postulated to contribute to their virulence. Consequently, we constructed an *engCP* Targetron mutant of *C. perfringens* and tested this mutant in the mouse myonecrosis model. The results showed that the *engCP* mutant was less virulent compared to its wild-type parent strain and that virulence could be restored by complementation *in trans* with the wild-type *engCP* gene. To examine its function, we demonstrated that purified EngCP could deglycosylate a-dystroglycan, a heavily *O*-glycosylated protein located in the sarcolemmal membrane of myofibres. However, EngCP did not appear to enhance membrane damage in *C. perfringens*-infected muscle tissue. Glycan array analysis of EngCP indicated that it could bind to glycan structures found on blood group antigens that are associated with immune cells, suggesting that EngCP may play a role in immunomodulation. These data provide evidence that EngCP is required for virulence in *C. perfringens* and indicate that although the toxins are important for disease, other factors also play a significant role in the disease process.

**110**

## Metabolic labelling and affinity-based protein profiling reveal putative transporters for the Pseudomonas Quinolone Signal

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*Pseudomonas aeruginosa* cells communicate with each other via the secretion and sensing of quorum sensing (QS) molecules, which collectively control the expression of multiple virulence factors. One of these QS molecules is the Pseudomonas Quinolone Signal (PQS), which is biosynthesized in the cytoplasm. Due to the hydrophobic nature of PQS, it is not freely diffusible across the aqueous periplasm separating the inner and outer membranes. However, the mechanisms by which PQS is transported across the cell envelope during secretion and uptake are not known. Here, we exploited metabolic labelling to introduce alkyne and diazirine functionalities into PQS during biosynthesis, and employed these bifunctional analogs for the profiling of PQS-binding proteins. We identified several membrane proteins that bind PQS, and showed that an ABC transporter and two RND efflux systems are functionally important for the uptake and secretion processes of PQS, respectively. Our work provides fundamental insights into PQS-mediated quorum sensing, and reveals potential targets for the development of novel anti-virulence agents.

**111**

## Unlocking the secrets of an ancient antimicrobial, honey, using modern transcriptomics

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Antibiotic resistance has been described as an 'apocalyptic' threat to human health. As resistance to antibiotics is common soon after they are introduced to clinical use, there is little investment in their development. The need for more treatment options has prompted interest in complex natural products as antimicrobials. Honey has been an effective topical wound treatment throughout history, predominantly due to its antimicrobial activity.

Honeys derived from different nectar sources exhibit vastly different levels of activity, and manuka honey has broad-spectrum antimicrobial activity effective against antibiotic resistant pathogens, such as ciprofloxacin-resistant *Pseudomonas aeruginosa,* and is currently licensed for use in honey-based wound dressings. Unlike traditional antibiotics, bacterial resistance to honey has not been reported, however honey remains underutilised in the clinic largely due to a lack of understanding of its mechanism of action.

Through passaging experiments, we found that honey resistance cannot be induced under conditions that rapidly induced resistance to antibiotics. We hypothesise that this low propensity for resistance is due to multiple modes of action unlike traditional single target antibiotics. We are currently investigating the mechanism of action of honey and its key components using a transcriptomic approach in a model organism, *P. aeruginosa*. Our results indicate that no single component of honey can account for its total antimicrobial action and that honey causes DNA and oxidative damage, and affects pathways involved in cell motility, central carbon metabolism, and quorum sensing. We found that honey uniquely affects the expression of the *P. aeruginosa* gene cassette encoding R-pyocins, also known as 'tailocins', which are involved in intraspecies competition and explosive cell lysis. Honey also downregulates the expression of various genes involved in maintaining the electron transport chain and causes protons to leak across biological membranes inducing their depolarisation. Taken together, these data argue that honey should be included as part of the current array of wound treatments, due to its effective antibacterial activity that does not engender bacterial resistance.

**112**

## A new versatile broad host range cloning and expression expression vector for Gram negative bacteria

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Heterologous expression is a key technique for understanding mechanisms of bacterial pathogenesis. Many cloning and expression vectors are available for Gram-negative bacteria but due to constant advances in methods (e.g. Golden-Gate cloning) and fundamental discoveries (e.g. new regulation systems), new and better plasmid tools are desirable.

Here we report a new plasmid vector (pUS250, 4.7 kb) that combines advantageous features of prior vectors with some novel elements to yield a highly versatile and efficient tool for gene cloning and expression. The plasmid is based on the broad host range KmR plasmid pBBR1MCS-2, which replicates in most Gram negative bacteria. The mobility functions of the original vector have been removed and replaced with a more space-efficient oriT sequence, and all 'junk' regions and annoying restriction sites in the plasmid backbone have been removed. The new plasmid features an extensive polylinker (26 unique sites) which is compatible with Golden Gate (BsaI and Esp3I) and BioBrick cloning. Cloning of a foreign gene into pUS250 results in excision of the amilCP chromoprotein gene, which enables blue/white selection of recombinants in any kind of bacterial host. Inducible expression of cloned genes occurs via a novel variant of the cumate control system from Pseudomonas putida, consisting of the cymR regulator and two CymR-binding operators flanking the strong Pc promoter from a class 1 integron.

We have shown that pUS250 can be mobilised between E.coli strains, and from E.coli to Pseudomonas and Rhizobium, confirming the functionality of the oriT. The cumate regulatory system is functional in all three hosts, and gives both tight control and also very high levels of derepressed expression; experiments with superfolder GFP as a test protein indicate similar performance to pET/BL21, but with the advantages that expression is not confined to E.coli, and is inducible by a very cheap and non-toxic substrate.

**113**

## Host mobilisation of zinc in response to pneumococcal infection

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Zinc deficiency is estimated to affect approximately one third of the global population. Zinc is critical for optimal host defence against infection, and consequently zinc deficiency is associated with increased morbidity and mortality for infectious diseases. *Streptococcus pneumoniae* (the pneumococcus) is a host-adapted pathogen and the most common cause of bacterial pneumonia in children under the age of 5 years. Bacterial pneumoniae is highly prevalent in regions that also have endemic zinc deficiency. In this study we sought to elucidate how dietary zinc deficiency could compromise host resistance to pneumococcal disease. We investigated how dietary zinc restriction in a murine model of *S. pneumoniae* infection impacted tissue zinc concentrations and infection kinetics. Here, we generated fluorescently labelled strains of *S. pneumoniae* for use in combination with elemental bio-imaging, a novel application of laser ablation-inductively coupled plasma-mass spectrometry. By combining fluorescence microscopy with elemental bio-imaging we reveal the spatial redistribution of zinc and show that it co-localised with the invading bacteria. Further, regions that were not enriched for zinc were devoid of bacteria. Taken together, these data show that the host mobilises zinc to sites of pneumococcal infection in lungs. Collectively, this approach shows how changes in the chemistry of the host environment changes can be mapped and will aid in elucidating how dietary zinc contributes to resistance against bacterial infection.

**114**

## CppA: A putative major fimbrial protein and novel virulence factor for clostridial myonecrosis?

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*Clostridium perfringens* is a Gram positive anaerobic bacterium that causes a variety of diseases ranging from mild diarrhoea to clostridial myonecrosis. Previous studies comparing the transcriptomes of *in vitro* cultures of a human gas gangrene isolate to equivalent cells from myonecrotic muscle tissues revealed that several genes were up-regulated in infected muscle tissues. Among these genes, *cppA* and *cppB* were shown to be upregulated by $\log_2$ (fold-change) of 4.30 and 5.02, respectively, suggesting that they may play a role in disease pathogenesis. To investigate their role in virulence, independent *ΔcppA, ΔcppB,* and *ΔcppAB* mutants were constructed by allelic exchange and examined in the mouse myonecrosis model. Wild-type disease levels were observed in mice infected with the *ΔcppB* and *ΔcppAB* mutants. However, mice infected

with the *ΔcppA* mutants were attenuated for virulence. These mice showed significantly improved survival compared to mice infected with the wild-type strain (log-rank Mantel-Cox test, $p < 0.0001$). Bioinformatic analyses suggested that these predicted CppA and CppB proteins may form fimbriae or may act independently as surface adhesins. Cell fractionation and Western blot analysis of a strain engineered to overexpress HA-tagged CppA showed that the CppA protein primarily localised to the membrane fraction. Stimulated emission depletion fluorescence microscopy on this strain showed that CppA is present on the surface of the bacterium. In conclusion, we have identified a novel surface protein, CppA, that appears to be required for virulence in *C. perfringens*-mediated clostridial myonecrosis.

**115**

## Enhancing group A streptococcal vaccine design through global population genomics and non-human primate infection studies.

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The development of a group A streptococcal (GAS) vaccine has been hindered by a number of hurdles including the high serotypic diversity of the pathogen, autoimmune complications following repeated GAS infections, and the lack of non-murine models to validate proposed GAS vaccine formulations. To overcome these hurdles, we generated a global GAS genome database to unravel the evolutionary dynamics of this major human pathogen. This database of 2,083 genomes were obtained from 22 countries, with a focus on sampling from streptococcal endemic settings. We identified a core panel of pre-clinical GAS vaccine antigens that would provide theoretical global coverage on the basis of >99% antigen carriage and <2% sequence heterogeneity. Using this platform, 5 conserved antigens (arginine deiminase [ADI], C5a peptidase [SCPA], streptolysin O [SLO], interleukin-8 [IL-8] protease [SpyCEP], and trigger factor [TF]), that have not been linked to autoimmune complications yet are highly conserved within a global context, were investigated as a putative multi-component vaccine formulation. We developed a non-human primate (NHP) infection model of GAS pharyngitis and evaluated the protective efficacy of the 5 conserved antigen formulation termed 'Combo5'. Antibody responses against all Combo5 antigens were detected in NHP serum, and immunised NHPs showed a reduced pharyngitis and tonsillitis compared to controls. Within an evolving global bacterial pathogen such as GAS, we have identified a number of proposed pre-clinical GAS vaccine antigens that fulfil the criteria for a global vaccine and provide protection from pharyngitis in a NHP model. Our work establishes a technical and experimental framework for the development of a global GAS vaccine.

**116**

## Bacteriophage isolation against common multidrug resistant human pathogens

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The use of bacteriophages, viruses that infect bacteria, in the treatment of multidrug resistant (MDR) infections is being increasingly regarded as an alternative to antibiotics. However, many questions regarding best clinical protocols are still debated. A promising clinical avenue is personalized therapy based on 'magistral' bacteriophage preparations that rely on the availability of large, well-characterized phage banks accessible ad hoc for patient care. For this purpose continued isolation and characterization of lytic phages against clinically relevant pathogens is critical. We have used modified standard methods for phage isolation to build an exhaustive phage library against MDR pathogens, in particular, but not exclusively, those related to severe infections (*E. coli*, *K. pneumoniae*, *S. aureus*, *Enterococcus ssp.*, *Pseudomonas aeruginosa*). To maximize isolation frequencies, our protocols included use of both enrichment and direct plating, varying agar concentrations for growth of phages that do not perform well under regular conditions, extended enrichment incubations, as well as multiple environmental sources for isolation (soil, water, wastewater, hair, skin, sputum, faeces). We isolated phages against common human pathogens ("high risk clones" *E. coli* ST131 and *K. pneumoniae* ST258, MDR *P. aeruginosa* and methicillin resistant and sensitive *S. aureus*) but with different rates of success and host specificities. For *K. pneumoniae* and *E. coli* we isolated diverse lytic phages, while for other bacterial species such as *S. aureus* standard isolation methods had poor success with frequent selection of temperate phages (not useful for immediate therapeutic applications). Differential results in phage isolation with standard methodology are dependent on the bacterial host genomic background, related to phage resistance mechanisms and species genomic variability, as well as to phage biology and bacteria-phage population dynamics. Bacteriophage isolation is usually not considered as a primary bottleneck for therapy, but this holds true only for a selected number of bacterial targets. There is a need for further study in order to improve isolation techniques to effectively select for virulent phages of clinical interest.

**117**

## Routine genomic surveillance to track treat and prevent Healthcare-associated infections

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Every year, between 165,000 and 200,000 Australians contract a healthcare-associated infection (HAI), causing significant ill health and costs to the health system. Furthermore, the impact of these infections is exacerbated by rapidly increasing rates of antibiotic resistance. Currently, there is limited laboratory capacity to track pathogens causing these infections in real-time or to detect cross-transmission events. However, low cost, whole genome sequencing (WGS) promises to revolutionise modern clinical microbiology by capturing the entire bacterial genome, providing unparalleled understanding of clinically relevant characteristics and transmission dynamics. Here, we established a pre-emptive WGS-based surveillance program to identify clustering of clinically relevant multi-resistant bacteria, suggesting in-hospital transmission, before an outbreak is established or recognised. Over a one year period (January 2018 to March 2019) more than 1000 multidrug-resistant bacterial isolates, specifically ESBL-producing Enterobacteriaceae, methicillin- resistance *S. aureus* (MRSA) and vancomycin-resistance enterococci (VRE), were collected from patients admitted to three metropolitan Brisbane hospitals, sequenced and subjected to genomic analysis. Core genome SNP data, used to establish the relationship between isolated and detect in-hospital transmission events, identified 28 distinct clusters (>2 isolates) of closely related isolates that were not identifiable using traditional surveillance techniques. Of these 28 clusters, 11 were contained to one of three target hospitals. The remaining 17 clusters represent inter-hospital transmission events or community outbreak strains acquired prior to hospital admission. Additional targeted environmental sequencing (isolate and metagenomics) of three clusters identified environmental reservoirs within the hospital as an important source of outbreaks of multi-drug resistant organisms. Overall, this work demonstrates that routine WGS surveillance is an effective tool to enhance and support established infection control responses. The single nucleotide resolution offered by WGS enables transmission pathways between patients and hospital environments to be delineated at the highest possible resolution and is essential for identifying missed outbreaks and excluding misidentified outbreaks, which can inappropriately trigger infection control responses.

**118**

## Biochemical and structural characterisation of the *Haemophilus influenzae* PsaA ortholog, HIPsaA

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*Haemophilus influenzae* is a host-adapted pathogen that colonizes the human nasopharynx that can mediate diseases of the upper and lower respiratory tract . *H. influenzae* has an array of molecular mechanisms that permit growth and virulence in diverse host niches. Notably, *H. influenzae* can respond to exogenous, host-mediated and endogenous, i.e. metabolically produced, reactive oxygen and nitrogen stresses. Defence against these chemical insults employs enzyme-mediated detoxification processes, such as the molybdenum-dependent HITorZ. Transition metal ions serve crucial roles in bacterial growth, survival and stress response, but the majority of these mechanisms in *H. influenzae* remain to be determined. Bioinformatic analyses of *H. influenzae* 2019 revealed that it encoded two ATP-binding cassette (ABC) transporter solute binding proteins (SBPs) that belonged to the cluster A-I subgroup. Primary sequence analyses suggested that these were orthologs of a manganese-specific (locus tag: C645_00940) and zinc-specific (locus tag: C645_02340) SBPs. Given the central role of manganese in metabolism and resistance to oxidative stress we investigated the biochemical and biophysical properties of C645_00940. We combined recombinant protein purification with *in vitro* metal binding assays to show that C645_00940 was a manganese-binding SBP. Building on this finding, the gene was renamed as HIPsaA due to the functional and structural similarity to *Streptococcus pneumoniae* PsaA. Collectively, this work provides insight into manganese acquisition in *H. influenzae* and the contribution of this transition metal ion to bacterial virulence.

**119**

## Understanding clonal expansions from a population genetics perspective

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Affordable and widely-available sequencing technology continues to generate data that can be used to detect new and ongoing outbreaks on a global scale, providing greater insight into particular bacterial populations. New tools should be developed to harness this influx of data and increase our resolution in monitoring the global expansions of infectious disease.

Recently, the population genetic statistic Fu's Fs identified a gene, *porA*, that was not only driving the expansion of a drug-resistant clone of *Campylobacter jejuni,* but was also necessary and sufficient for causing abortion in sheep (Wu, Z. et al. 2016). This analysis was applied after bacterial sexual genetics in a guinea pig model had also identified *porA* and suggested that population genetic statistics could be used to elucidate particular loci driving an outbreak.

We will use pandemic *Escherichia coli* ST131, a clone associated with high antibiotic resistance, as an independent validation. Multiple hypotheses have been generated to explain the success of this well-studied clone, making it a perfect candidate for our analysis. We believe that, in the process of understanding the use of these population genetic statistics for bacteria, we can also identify specific loci under selection for ST131 in an unbiased manner.

I will discuss Fu's Fs and related statistics applied to *E. coli* ST131, and the resulting preliminary data and challenges. For instance, horizontal gene transfer and recombination, diversity mechanisms common in bacteria, violate assumptions for some of these statistics. As these methods have

rarely been applied to large bacterial datasets, we must also understand how the diversity and size of the studied population affect our ability to detect these evolutionary signals.

This project will provide a set of new approaches for understanding bacterial genomic data, improving our understanding of infectious disease and its spread on a global and local scale.

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**120**

## Structural and Functional Analysis of a Representative PACE protein

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Bacterial antimicrobial resistance is recognised as one of the greatest challenges currently facing human health, and the threat it poses is continuing to grow. To combat this issue, there is an urgent need for increased research into the wide array of mechanisms through which bacteria resist antimicrobials. One of the key contributors to antimicrobial resistance are multidrug efflux pumps, which are able to recognise a diverse range of antimicrobials and transport them out of the bacterial cell, preventing them from reaching and acting on their targets and leading to resistance. The proteobacterial antimicrobial compound efflux (PACE) family is the most recently discovered group of multidrug efflux pumps, and has been implicated as a contributor to resistance in a number of key multidrug-resistant human pathogens, such as *Acinetobacter baumannii.*

The *Vibrio parahaemolyticus* protein VP1155 is a prototypical member of this family that is capable of transporting several chemically distinct antimicrobial compounds and fluorescent dyes. VP1155 was used as a representative PACE protein to investigate the topology and oligomeric state of PACE proteins, as well as the functional importance of charged residues that are highly conserved within the PACE family. The results of native PAGE analysis conducted with protein solubilised in SMA lipid particles suggested that VP1155 forms multimers within the membrane, and that these are most likely to be tetramers. Site-directed mutagenesis and fluorescent transport studies indicated that specific conserved charged residues at the cytoplasmic boundary of the membrane were required for antimicrobial transport function. These results highlight structural and mechanistic features that are unique to the PACE family of transport proteins.

**121**

## Exploring gene essentiality in Klebsiella pneumoniae

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*Klebsiella pneumoniae* is a key nosocomial pathogen associated with increasing resistance to first-line antibiotics. Therefore, the identification of novel targets is required for the treatment of infections caused by *K. pneumoniae*. Genes that are essential for the survival of a pathogen but not a commensal organism might represent good targets. We have used Transposon Directed Insertion-site Sequencing (TraDIS) to generate a highly-saturated mutant library of *K. pneumoniae* ECL8. The library consists of 476,249 unique transposon insertion sites which represents an average insertion every 10 base pairs throughout the genome. To define whether a gene is essential, the number of transposon insertions normalised for coding sequence (CDS) length was calculated and denoted the insertion index score (IIS). The genome-wide IIS distribution was bimodal. For a given insertion index score, the probability of the gene belonging to the essential or non-essential mode was calculated, and the ratio of these values was represented as a log likelihood score. A gene was classified as essential if its log likelihood score was less than $\log_2 12$ and was therefore 12 times more likely to belong to the essential mode than the non-essential mode. A total of 376 *K. pneumoniae* genes satisfied these stringent criteria and were therefore identified as essential. Analysis of the clusters of orthologous groups (COGs) of the essential genes showed that gene products related to translation, cell wall biogenesis and coenzyme metabolism were enriched. Comparison between the essential genes of *E. coli* K-12 and *K. pneumoniae* has revealed key differences between these two organisms. *E. coli* and *K. pneumoniae* share 272 essential genes, however we have identified 103 genes that are essential for *K. pneumoniae* only. These 103 genes represent potential novel candidates for the targeted treatment of *K. pneumoniae* infections.

**122**

## Genetic Basis and Regulatory Control of IncC Plasmid Conjugation

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IncC plasmids are a medically important group of broad-host-range plasmids that facilitate the spread of antibiotic resistance genes in human enteric pathogens. In particular, these plasmids are linked to the emergence of carbapenem-resistant *Enterobacteriaceae* and their carriage of genes encoding metallo-β-lactamases with carbapenemase activity.

Despite their impact on antibiotic resistance, our understanding of the genetics of IncC-conjugation is limited. Here, we utilised hyper-saturated transposon mutagenesis coupled with transposon directed insertion site sequencing (TraDIS) to determine the set of genes required for IncC-conjugation. Overall, 27 genes were identified, including all 17 predicted and known conjugation genes, two regulatory genes (*acaDC)* and eight genes not previously associated with conjugation. Targeted mutagenesis of the eight novel genes confirmed a significant role for five of these genes in conjugation.

We show that one gene, *acaB*, encodes a novel regulator that plays a central role in IncC regulation circuitry. AcaB activates conjugation via binding to a 13bp sequence upstream of the *acaDC* promoter and upregulates *acaDC* transcription, a controlling element that in-turn activates transcription of genes involved in IncC-conjugation. The crystal structure of AcaB revealed a new protein fold belonging to the ribbon-helix-helix (RHH) superfamily, based on two β-α-α units folding into a 4-helix bundle and an exposed two-stranded b-sheet. Functional analyses guided by these structural studies determined that the mechanism of AcaB binding to DNA occurs via the β-sheet region.

Overall, we have defined the specific regulatory mechanism of a novel element that is central to the control of conjugative transfer of IncC multidrug resistance plasmids.

**123**

## DNA contributes to the stability of biofilms formed by *Klebsiella pneumoniae* AJ094

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*Klebsiella pneumoniae* is a leading cause of nosocomial infections, manifesting as a variety of diseases including pneumonia, urinary tract infections and septicemia with a high mortality rate particularly in immune-compromised patients. The formation of biofilms is a key virulence mechanism in *K. pneumoniae* providing the bacterium with increased resistance to antimicrobial treatments. Our analysis of *K. pneumoniae* strains revealed large differences in biofilm formation that were independent of *mrk* operon carriage (*mrk* encodes type 3 fimbriae) and capsule type. We hypothesized that extracellular DNA (eDNA) forms a major component of biofilm structure. To determine whether removing eDNA affects biofilm formation by *K. pneumoniae* AJ094, a strain with an enhanced capacity to form biofilms, was studied. Biofilm formation was quantified using a microtiter plate biofilm assay with crystal violet staining. Bacteria were treated with various concentrations of DNase either at the time of inoculation for a period of 8 hours, or to established biofilms after 24 hours of incubation. Biofilms containing bacterial cells and eDNA were visualized by confocal microscopy using the fluorescent dyes SYTO-60 and TOTO-1. DNase treatment (as low as 2.5 µg/mL) reduced early-phase biofilm formation by more than 50%, demonstrating the importance of eDNA in initial biofilm stability. Conversely, established biofilms were not disrupted following DNase treatment. The excretion of eDNA by bacteria residing in biofilms was demonstrated using confocal microscopy. In conclusion, eDNA secretion or release represents a target to prevent *K. pneumoniae* biofilm formation.

**124**

## Surfaceome analysis of Australian epidemic *Bordetella pertussis* reveals potential vaccine antigens

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*Bordetella pertussis* causes whooping cough. The predominant strains in Australia changed to single nucleotide polymorphism (SNP) cluster I (pertussis toxin promoter allele *ptxP3/*pertactin gene allele *prn2*) from SNP cluster II (non-*ptxP3*/non-*prn2*). SNP Cluster I was mostly responsible for the 2008–2012 Australian epidemic. Our previous proteomic analysis identified potential proteomic adaptations in the whole cell and secretome of SNP cluster I. Additionally, current ACVs were shown to be less efficacious against SNP cluster I in mice models and there is a pressing need to discover new antigens to improve the ACV. One important source of novel antigens is the surfaceome. Therefore, this study established surface shaving in *B. pertussis* to compare the surfaceome of SNP cluster I (L1423) and II (L1191), and identify novel surface antigens for vaccine development. Surface shaving using 1 µg of trypsin for 5 min identified 126 proteins from 666 peptides detected with the most abundant being virulence-associated and known outer membrane proteins. Cell viability counts showed minimal lysis from shaving. The proportion of immunogenic proteins was higher in the surfaceome than in the whole cell and secretome. Of the 126 proteins, 6 were unique to L1423 including two transport proteins and 2 were unique to L1191, both of which are known to be immunogenic. Two type III secretion system proteins also known to be immunogenic were downregulated in L1423, providing further evidence of proteomic adaptation in SNP cluster I. Finally, a comparison of proteins in each sub-proteome identified 22 common proteins. These included 11 virulence proteins and 11 housekeeping proteins which were immunogenic, essential and consistently expressed thus demonstrating their potential as future targets. This study established surface saving in *B. pertussis* and identified unknown surface proteins which may be potential vaccine antigens.

**125**

## Genomic epidemiology of erythromycin-resistant *Bordetella pertussis* in China

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Erythromycin is the empirical treatment of *Bordetella pertussis* infections. China has experienced an increase in erythromycin-resistant *B. pertussis* isolates since they were first reported in 2013. Here, we undertook a genomic study on Chinese *B. pertussis* isolates from 2012 to 2015 to elucidate the origins and phylogenetic relationships of erythromycin-resistant *B. pertussis* isolates in China. A total of 167 Chinese *B.*

*pertussis* isolates were used for antibiotic sensitivity testing and multiple locus variable-number tandem repeat (VNTR) analysis (MLVA). All except four isolates were erythromycin-resistant and of the four erythromycin-sensitive isolates, three were *non-ptxP1*. MLVA types (MT), MT55, MT104 and MT195 were the predominant types. Fifty of those isolates were used for whole genome sequencing and phylogenetic analysis. Genome sequencing and phylogenetic analysis revealed three independent erythromycin-resistant lineages and all resistant isolates carried a mutation in the 23S rRNA gene. A novel *fhaB3* allele was found uniquely in Chinese *ptxP1* isolates and these Chinese *ptxP1-ptxA1-fhaB3* had a 5-fold higher mutation rate than the global *ptxP1-ptxA1 B. pertussis* population. Our results suggest that the evolution of Chinese *B. pertussis* is likely to be driven by selection pressure from both vaccination and antibiotics. The emergence of the new non-vaccine *fhaB3* allele in Chinese *B. pertussis* population may be a result of selection from vaccination, whereas the expansion of *ptxP1-fhaB3* lineages was most likely to be the result of selection pressure from antibiotics. Further monitoring of *B. pertussis* in China is required to better understand the evolution of the pathogen.

**126**

## Polymicrobial infection and neutrophilic disease in cystic fibrosis airways

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Cystic fibrosis (CF) lung damage is driven by a cycle of infection, pro-inflammatory signalling, and neutrophilic reprogramming. However, the mechanisms behind this process are poorly characterized. We created a multi-component *in vitro* system to model CF inflammatory responses and airway neutrophil recruitment, specifically in the context of polymicrobial infections. This was used to assess epithelial and neutrophil responses to rhinovirus and *Pseudomonas aeruginosa* infection. Submerged monolayers of primary CF airway epithelial cells (3M:1F; age≤5yr) were infected individually and in combination with rhinovirus strain RV1b (MOI 0.5) and a mucoid *P. aeruginosa* clinical isolate (MOI 0.001). After 48 hours, cell culture supernatants were harvested and epithelial secreted cytokines quantified by ELISA. Filtered supernatants were also applied to an *in vitro* model of neutrophil transmigration to the airways. Migrated neutrophils were harvested 10 hours post stimulation and assessed by flow cytometry. Both infection with RV1b or RV1b+P. aeruginosa significantly increased production of proinflammatory cytokines IL-8 and IL-1β compared to uninfected controls or bacterial infection alone ($p<0.01$). Production of CCL5 was significantly increased in viral infections ($p<0.03$). Biofilms formed upon *P. aeruginosa* infection, however, more non-aggregated planktonic bacteria were observed with RV1b+*P. aeruginosa* coinfection. In the transmigration model, neutrophils migrated in similar numbers towards all supernatants. However, neutrophils migrating towards bacterial or coinfection supernatants had significantly reduced staining of CD16, a phagocytosis marker ($p<0.01$). Expression of exocytosis marker CD63 was unchanged. Results highlight the role of respiratory viruses in CF as triggers of airway inflammation and promoters of secondary bacterial infection. Coinfection induced the greatest change in expression of a neutrophil phagocytosis marker, suggesting that polymicrobial infections may be implicated in CF neutrophilic reprogramming. This model permits investigation of coordinated CF airway responses to diverse pathogenic insults. Ongoing work will assess responses in non-CF airway epithelium and discern how recruited neutrophil signalling further enhances reprogramming.

**127**

## Transposon-Insertion Sequencing reveals the genetics underpinning bacterial tolerance of common hospital and household biocides

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Biocides, such as antiseptics and disinfectants, are used ubiquitously for hygiene in households and for life-saving infection control in hospitals. An increasing concern is the widespread use of biocides may contribute to the emergence and spread of multidrug-resistant bacteria. For the first time, we performed transposon directed insertion site sequencing (TraDIS) to identify genes or key cellular pathways of the multidrug resistant nosocomial pathogen *Acinetobacter baumannii* affecting host fitness during exposure to a panel of ten structurally-diverse and clinically-relevant biocides: silver nitrate, benzalkonium, cetyltrimethylammonium bromide (CTAB), chlorhexidine, triclosan, chloroxylenol, polyvidone iodine, bleach, glutaraldehyde and ethanol. We identified a number of electron transport genes as contributing to tolerance of most biocide treatments, and showed that these biocides caused a drop in cell membrane potential even at sub-inhibitory concentrations; based on these findings, we propose that collapse of membrane potential is a common direct or downstream biocidal mode of action. In addition, the core antibiotic efflux systems in *A. baumannii*, i.e. AdeABC and AdeIJK, confer resistance to half of the biocides, suggesting these compounds have the potential for co-selection of resistance to the multiple antibiotics that are also transported by these pumps.

**128**

## The Gram-negative bacterial periplasm; Architecture significance

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Here we investigated the key player in the bacterial cell envelope organization; a small, alpha-helical lipoprotein protein Lpp, AKA Braun's lipoprotein. Lpp provides the only covalent connection between the outer membrane (OM) and the peptidoglycan (PG). The N-terminus is attached to the OM while the C-terminus is attached to the PG. Recent work has shown that the width of the periplasm is controlled largely by Lpp. Lengthening of Lpp allowed the expansion of the periplasm relative to the number of heptad repeats inserted within the Lpp sequence.

The bacterial cell envelope is the interface through which bacteria interact with their environment, everything going into or out of the cell must transit it, and many important cellular functions take place within its enclosed periplasm: cell division regulation, osmoregulation, peptidoglycan synthesis, multidrug efflux systems, and many others. we sought to use a synthetic lethal genetic screening approach to identify periplasmic processes that are dependent on the periplasmic architecture. In addition, RNAseq and proteomics studies were performed on the lengthened Lpp isoform. Here we report the effect of a widened periplasm on peptidoglycan assembly. The activation of the major penicillin binding proteins (PBP1A and PBP1B) by their cognate lipoproteins (LpoA and LpoB) is compromised which leads to synthetic lethal phenotype when either of the redundant pathway is removed. We also show the significance of OmpA, Pal, and TolC in non-covalent binding of the PG. The data also shows compromised iron import and lipopolysaccharide (LPS) trafficking across the periplasm. These genetic screen results are further studied to better understand the coordination between the OM and PG biogenesis in bacterial cell elongation.

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**129**

## *Escherichia coli* from companion animals, livestock, wildlife and food as potential sources of antimicrobial resistance and virulence associated genes

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Antimicrobial resistance (AMR), particularly against critically important antimicrobials (CIA), has been recognised as one of the world's most pressing public health problems. The presence of AMR determinants in different environments is of concern and likely contributes to AMR maintenance and diffusion.

Illumina whole genome sequencing and a range of bioinformatic tools were employed to investigate 300 *Escherichia coli* isolated from 12 sources (dairy, beef, wild boar, rabbit, poultry, swine, companion animal, vegetable, fishery, mollusc, wild animal and human) in Italy. We evaluated phylogenetic diversity, presence of antimicrobial resistance genes (ARGs), virulence-associated genes (VAGs), disinfectant resistance genes and their association with mobile genetic elements (MGEs).

Poultry and rabbit meat were the most worrisome sources, showing an extremely high number of both ARGs and VAGs. Also 3 genes, usually associated with mobile genetic elements and coding for quaternary ammonium compound resistance, were identified. The presence of both ExPEC and IPEC VAGs in these strains indicated the potential presence of hybrid pathogens.

Mobile colistin resistance gene *mcr1* was identified in rabbit meat and intestine samples in association with IS6 family elements. STs commonly associated with human infections were identified among poultry strains.

Dairy strains carried the most extended spectrum beta lactamases (ESBL) genes. Companion animals, living in close contact with humans, harboured the highest number of VAGs among the collection. Interestingly, wild animals and wild boar showed a wide variety and a relatively high rate of different virulence determinants, suggesting their hypothetical role as a reservoir of VAGs.

Different environments (food chain, food-producing and companion animals, wildlife) have been identified as possible reservoirs of ARGs and/or VAGs and, ultimately, of hypothetical pathogens for humans. Understanding the role of different sources in the maintenance and diffusion of AMR represents an essential step for preventive measure implementation and epidemiological evaluation.

**130**

## *Vibrio cholerae* in food vacuoles expelled by protozoa are protected from stresses and more infectious *in vivo* than free-living cells

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*Vibrio cholerae* is an aquatic bacterium that is the aetiologic agent of the acute diarrhoeal disease cholera, which is endemic in many countries. In aquatic environments, *V. cholerae* interacts with a wide variety of organisms, including heterotrophic protists (protozoa). Several species of these bacterial predators have been reported to release live, undigested bacteria in expelled food vacuoles (EFVs) when feeding on certain pathogens. While the production of EFVs has been reported, their biological role as a vector for the transmission of pathogens remains unknown.

Using co-incubation assays, we report that *Tetrahymena pyriformis* releases large numbers of EFVs when feeding on *V. cholerae*. The EFVs are stable, the bacterial cells within are protected from multiple stresses (low pH, antimicrobials and starvation) and vast numbers quickly escape when incubated at 37°C or in the presence of nutrients. We show that OmpU, a major outer membrane protein positively regulated by ToxR, plays a significant role in the production of EFVs. Importantly, cells released from EFVs have growth and colonisation advantages over planktonic cells both *in vitro* and *in vivo* and are highly infectious (as shown in the infant mouse model of infection). Our results suggest that EFVs facilitate *V. cholerae* survival in environment and in the gastric environment, enhancing infectious potential and may significantly contribute to the dissemination of epidemic *V. cholerae* strains. These results establish a new understanding of the mechanisms of persistence and the modes of transmission of *V. cholerae* and may further apply to other opportunistic pathogens that have been shown to be released by protists in EFVs. Results presented here will improve the identification and tracking of pathogens in the environment.

**131**

## Global transcriptional profiles in RND efflux pump mutants

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

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

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**132**

## Modelling natural immunity to *Streptococcus pyogenes* skin and mucosal infections in a mouse model

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*Streptococcus pyogenes* is a Gram-positive bacterial pathogen of humans. It causes a broad range of disease from self-limiting throat and skin infections, to life-threatening streptococcal toxic shock syndrome and rheumatic heart disease resulting in over 500 000 deaths annually. Global efforts have been aimed at the development of a vaccine, but large serotypic diversity and the potential for autoimmune cross-reactivity have impeded this development. Current vaccine efforts are yet to define a single vaccine to protect against both skin and mucosal infections. In humans, natural immunity is slow to develop and its role in preventing *S.pyogenes* infections is poorly understood. In addition, there is a need to understand the mechanism of cross-compartment immunity in *S.pyogenes* infections to aid in guiding vaccine development to protect from multiple serotypes and infection sites.

Through multiple sequential infections, we modelled repeated natural exposure to *S.pyogenes* in mice. We analysed bacterial load following each infection and screened for antibodies and antibody-secreting cells using ELISA and ELISPOT respectively. Using cytokine analysis and flow cytometry we aim to dissect the immune mechanisms involved in the development of immunity.

Following four homologous infections, while no circulating antibodies were detected, an increase in antibody secreting cells compared to naïve mice was observed. While assessing bacterial load, we determined that multiple sequential infections using a homologous isolate result in not only site-specific protection but also cross-compartment protection. Ongoing studies will investigate the effect of sequential heterologous infections on the development of immunity to multiple *S.pyogenes* strains.

**201**

## A novel method of O-antigen-specific antibody purification

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Historically antibody has been associated with improved host clearance of bacteria, although within the last decade a novel subset of antibody was found to inhibit complement-mediated killing in patients with chronic Gram-negative infections. This "inhibitory" antibody was found to be IgG2 specific to O-antigen, and was correlated with impaired disease in *P. aeruginosa*-infected patients with bronchiectasis [1]. As such, a novel salvage therapy for untreatable *P. aeruginosa* infections in two patients with bronchiectasis was conducted, known as plasmapheresis. This treatment involved total removal of antibody resulting in greatly improved patient disease symptomology and outcomes [2]. However this therapy is imprecise requiring removal of potentially protective antibody, highlighting the need for refinement of this therapy to produce a column that selectively removes inhibitory antibody. In addressing this, lipopolysaccharide from *P. aeruginosa* was purified using hot-phenol water extraction, where O-polysaccharide was separated from the lipid-A:core component via acetic-acid hydrolysis. O-antigen from varying serotypes (O3, O5, O6 and O11) was biotinylated at the Kdo residue of the terminal polysaccharide, and affixed to 1 mL streptavidin-sepharose columns. Chronically infected patient serum was applied to the column, and subsequent flowthrough and eluted antibody was tested for the ability to inhibit serum killing of cognate bacterial isolates *in vitro*. O-antigen columns were able to separate antigen-specific polyclonal antibody from sera across all tested serotypes showing no-detectable signs of O-antigen-specific antibody in flowthrough. In turn, normal serum killing was restored in patient serum depleted of O-antigen specific antibody and conversely, O-antigen-specific antibody in saline at physiological titres inhibited serum killing from cognate *P. aeruginosa* serotypes ($P < 0.05$). These results reinforce the mechanism of inhibitory antibody in complement-mediated killing and also provide a medium to purify O-antigen specific antibody targeting a range of O-serotypes. More promisingly, this technique of antibody purification also provides a preliminary means to develop novel therapies that involve removal of inhibitory antibodies from chronically-ill patients.

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**202**

## The role of lactate metabolism in *Acinetobacter baumannii* pathogenesis

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*Acinetobacter baumannii* is a Gram negative, obligate aerobe, coccobacillus and one of the most prevalent causes of nosocomial infections. With only a limited number of "traditional" virulence factors, the mechanisms contributing to the success of this pathogen are of increasing interest to researchers and clinicians alike. During previous investigations we have shown the putative lactate metabolism operon is significantly upregulated during an *in vivo* systemic infection. Therefore, to understand the contribution of lactate metabolism to bacterial physiology and pathogenesis, we investigated the role of the lactate permease, *lldP,* in the contemporary *A. baumannii* clinical strain AB5075-UW, using a transposon disrupted mutant acquired from the Manoil *A. baumannii* mutant library. As part of our study we have engineered two plasmid vectors for complementation, through modification of the *A. baumannii* expression vector pBASE, to encode either tellurite or hygromycin resistance cassettes, respectively. Using a multifaceted approach, combining traditional microbiology, molecular techniques and virulence assessments we have confirmed *lldP* is responsible for lactate metabolism *in vitro.* In addition, we have shown that the elevated expression observed previously *in vivo* is not strain specific, and is a phenotype conserved across multiple strains, whereby disruption of this pathway results in attenuation during a murine systemic *in vivo* competition assay. Our study provides both molecular resources for the *Acinetobacter* community in the form of two new expression vectors for use with multidrug resistant *A. baumannii* strains, in addition to advancing our understanding of the molecular and biochemical mechanisms responsible for the success of this pathogen *in vivo*.

**203**

## Uncovering the mechanisms of resistance in a multi-drug resistant *Acinetobacter baumannii* isolate

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*Acinetobacter baumannii* has recently been identified by the World Health Organisation (WHO) as one of the top three groups of bacteria for which new antibiotics is needed urgently. *A. baumannii* is a hospital-acquired opportunistic pathogen, which is now considered a threat globally, mainly due to the persistent nature of this pathogen and its propensity to acquire antimicrobial resistance phenotypes at unforeseen rates.

The aim of this study was to use a recent multi-drug resistant isolate of *A. baumannii* to understand the complex cellular responses of this pathogen to several classes of antimicrobials. A panel of 20 antimicrobials were chosen, these included several classes of antibiotics; disinfectants and antiseptics. Changes in expression at both the protein and transcript levels in response to antimicrobial stress were studied using RNA-sequencing, mass spectrometry (SWATH-MS) and high-throughput phenotypic screening using a transposon mutant library.

While each antimicrobial stress showed a unique expression profile, clustering pattern of genes in response to the panel of antimicrobials revealed overlaps in gene expression, therefore suggesting similar mechanisms of antimicrobial resistance. For example, overlaps in expression were evident in several efflux pump genes, motility genes and iron acquisition systems. We further investigated the broad-spectrum antibiotic, Ciprofloxacin. The bactericidal action of Ciprofloxacin is well studied in other organisms, wherein it is known to inhibit enzymes including topoisomerases. The most highly upregulated genes in the Transcriptomics and Proteomics data for Ciprofloxacin stress were genes associated with the SOS response and phage genes. The next most highly up-regulated gene in Ciprofloxacin stress was a hypothetical protein of unknown function. Disruption of this hypothetical protein in *A. baumannii* renders it more susceptible to Ciprofloxacin. Additionally, orthologs of this hypothetical protein is conserved in the core genome of *Acinetobacter* and is encoded in the genome of a range of Proteobacterial species. Collectively, these preliminary data show a novel gene which confers resistance to the antibiotic Ciprofloxacin in *Acinetobacter baumannii*.

**204**

## Exploring the therapeutic potential of phage therapy to treat *Pseudomonas aeruginosa* infection in people with cystic fibrosis

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Cystic fibrosis (CF), a genetic disease caused by mutations in the CFTR gene, leads to mucus build-up in the lungs of patients, creating an ideal environment for the growth of *Pseudomonas aeruginosa* (*P. aeruginosa*). Most CF patients will be persistently colonised by *P. aeruginosa* by the time they reach adulthood. *P. aeruginosa* infections are associated with a rapid decline in pulmonary lung function and increased risk of morbidity and mortality. Importantly, *P. aeruginosa* harnesses multiple intrinsic virulence factors that allows immune response evasion and resistance to antimicrobials. Chronic infection promotes further acquisition of resistance genes via adaptive mutations. In addition, *P. aeruginosa* forms biofilms which limits efficacy of antibiotic treatment. In this study, we seek to explore alternative treatments targeting *P. aeruginosa* infection in CF lungs, specifically using bacteriophages, or "phages" as a novel antimicrobial therapy. Over 75 environmental water samples were collected from freshwater ponds located around the Perth metropolitan area. Samples were filtered with 0.22µm filters to remove the presence of environmental microorganisms present within the ponds. Filtrates were then assessed by plaque assay for presence of *P. aeruginosa* specific phage using strain PA01 (ATCC 15692). Phage isolates exhibiting anti-*P. aeruginosa* activity were propagated from at least 4 water samples collected. These isolates were further characterized for their stability and lytic capabilities as well as their ability to clear *P. aeruginosa* in relevant infection models. Here, primary airway epithelial cells from children with CF will be cultured at air liquid interface to form a differentiated epithelial layer, then inoculated with biofilm forming *P. aeruginosa* and treated with phage. The effect of phage treatment will then be assessed by measuring bacterial load and epithelial inflammatory cytokine production. Generated results will give insights into the efficacy of phage therapy in CF with the potential to develop a novel therapeutic pipeline to help treat CF bacterial lung infections.

**205**

## Complex multi-level control of hemolysin production by uropathogenic *Escherichia coli*

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Uropathogenic *Escherichia coli* (UPEC) are the major cause of urinary tract infections. Nearly half of all UPEC strains secrete hemolysin, a cytotoxic pore-forming toxin. Here, we show that the prevalence of the hemolysin toxin gene (*hlyA*) is highly variable among the most common 83 *E. coli* sequence types represented on the EnteroBase genome database. To explore this diversity in the context of a defined monophyletic lineage, we contextualized sequence variation of the *hlyCABD* operon within the genealogy of the globally-disseminated multidrug-resistant ST131 clone. We show that sequence changes in *hlyCABD* and its newly defined 1.616-kb long leader sequence correspond to phylogenetic designation, and that ST131 strains with the strongest hemolytic activity belong to the most extensive multidrug resistance sublineage (clade C2). To define the set of genes involved in hemolysin production, the clade C2 strain S65EC was completely sequenced and subjected to a genome-wide screen by combining saturated transposon mutagenesis and transposon-directed insertion site sequencing with the capacity to lyse red blood cells. Using this unique approach, and subsequent targeted mutagenesis and complementation, 13 genes were confirmed to be specifically required for production of active hemolysin. New hemolysin controlling elements included discrete sets of genes involved in LPS inner-core biosynthesis (*waaC*, *waaF*, *waaG*, *rfaE*) and cytoplasmic chaperone activity (*dnaK*, *dnaJ*), and we show these are required for hemolysin secretion. Overall, this work provides a unique description of hemolysin sequence diversity in a single clonal lineage and describes a complex multi-level system of regulatory control for this important toxin.

**206**

## VirY, a small regulatory RNA that regulates toxin production in *Clostridium perfringens*

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*Clostridium perfringens* causes clostridial myonecrosis or gas gangrene in humans. *C. perfringens* produces many toxins and enzymes that aid in destroying human tissues and acquiring nutrients. To coordinate virulence-related gene expression, the VirSR two-component regulatory system regulates the *vrr* gene, which encodes a small regulatory RNA (sRNA) called VR-RNA. This sRNA controls the expression of many genes, including α-toxin gene, *plc*and κ-toxin gene, *colA*. However, detail of the mechanism of regulation is not clear, and it has been thought that more complicated regulatory networks are also involved.To identify a new putative regulator, a chromosome library was constructed and used to transform a gas gangrene isolate, strain 13. One transformant showed stronger α-toxin activity. Northern blot analysis showed that this strain had increased transcription of *plc* and *colA*. Sequence analysis showed that the insert encoded the *CPE0205* gene and a sRNA, which was designated as VirY. Both *CPE0205* and VirY were found to activate *plc* and *colA*, with a *CPE0205* and VirY-double mutant showing less α-toxin activity than the wild type, but not as reduced as a VR-RNA mutant. Interestingly, expression of *CPE0205* and VirY were found to be affected by VirSR. Network analysis using Microarray data performed under different culture conditions verified that VirY was involved in a complex network associated with toxin gene regulation. Although *CPE0205* and VirY were demonstrated to regulate *plc* and *colA* transcription, the precise mechanism of regulation is as yet unknown. Work is currently underway to elucidate this novel regulatory mechanism.

**207**

## Characterization of *Escherichia coli Adhesins*

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Uropathogenic *Escherichia coli* (UPEC) are the main causative agent of urinary tract infections (UTI), which are one of the most common infections in humans. Roughly 175 million cases of UTI are estimated to occur annually across the globe, accounting for more than 1 million hospitalizations and approximately $3.5 billion in medical expenditure each year in the USA alone. UPEC possess an array of virulence factors that mediate colonization of the urinary tract and enhance disease progression, including fimbriae. In this study, our aim was to characterize one type of UPEC fimbriae referred to as Yad fimbriae. Yad fimbriae contribute to bladder colonization, but the mechanism by which this occurs remains to be elucidated. To characterize Yad fimbriae, we first performed an *in silico* analysis on a collection of 597 completely sequenced *E. coli* genomes to assess the prevalence and sequence conservation of genes in the *yad* operon. We found that the *yad* genes were present in 74% of these strains and clustered into four major clades. *In silico* analysis was performed for individual fimbrial elements including the major pilin, usher and tip adhesin. We found that the tip adhesin exhibits greater sequence variation than the major pilin and usher proteins. Hence, we focused on the characterization of tip located adhesin protein (YadC) of Yad fimbriae. To study the adhesive characteristics of YadC, the region encoding the lectin binding domain was cloned from strains representing each major Yad clade and expressed in an isogenic recombinant *E. coli*background. The recombinant YadC proteins were purified by Ni-affinity chromatography under native conditions their receptor binding profile was assessed using a glycan array. Evaluation of this data is ongoing.

**208**

## Does quorum-sensing regulated by iron in *Klebsiella pneumoniae*?

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*Klebsiella pneumoniae* is a Gram-negative bacteria responsible for causing a variety of infections, such as pneumonia, hepatic abscess, meningitis, urinary tract infection among others. In recent years, an increase in resistance to antibiotics has been observed for some strains of *K. pneumoniae*. In addition, increasing cases of severe infections in healthy subjects have emphasized the importance of studying the mechanisms of virulence that determine the pathogenicity of *K. pneumoniae.* The communication between bacteria denominated quorum-sensing is an important mechanism of virulence factors. This communication happens through self-inducers molecules codified and captured by *luxS/luxI* and *luxR*, respectively. Another important component responsible to regulate the expression of innumerate virulence factor pathogenic bacteria is the iron. This regulation is mediated by the Fur transcriptional regulator which, when complexed to iron, binds to specific sequences, called Fur boxes, located in the promoter region of the target genes, leading to the repression or induction of the transcription of these genes. Bioinformatics analyses were used to identify genes homologous to *luxR* genes in the *K. pneumoniae* genome. For these analyses the nucleotide and amino acid sequences of the *luxR* genes of other Gram-negative bacteria were used. Identification of the homologous genes was performed on the genome of *K. pneumoniae* MGH78578 annotated in the NCBI, using the NCBI BLAST program. Moreover, genes homologous to *luxR* identified had their promoter region searched for the identification of probable Fur boxes. The results showed eight homologous genes to *luxR*, besides the already known *sdiA*. The amino acid sequences of the proteins encoded by genes homologous to *luxR* indicate helix-turn-helix (HTH) DNA binding domains, which are typical of LuxR-type regulators. In addition, Fur boxes were identified in the promoter or inside region these homologous, as well as in *sdiA* and *luxS* gene, identified. These results reveal the possibility of the quorum-sensing mechanism being regulated by iron levels in *K. pneumoniae.*

**209**

## Study on the role of O-antigen modifying glucosyltransferase (*gtr*) genes in *Shigella flexneri* serotype 1c virulence

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Shigellosis have continued to be a major cause of childhood death under the age of five, mostly in the developing countries. Among four species of *Shigella*, *S. flexneri* is the primary cause of Shigellosis in the developing countries. *S. flexneri* serotype 1c is a novel serotype first isolated in Bangladesh in 1980s and since then it has been isolated and identified in different parts of the world. *S. flexneri* serotype 1c possess highly complex O-antigen structure modified by three different bacteriophages. Several bacteriophages encoded virulence factors have been identified that play roles in different stages of the bacterial pathogenesis including toxin production, host epithelial cell invasion, adhesion, intracellular survival and antigenicity. In this study, we plan to investigate the role of O-antigen modifying genes-*gtrI* and *gtrIC* genes in *Shigella* virulence. We knocked out these two genes using lambda red mediated recombinase approach. The mutants and the wild type strain were used to examine the role of these genes in virulence using *in vitro* assays such as plaque and invasion assays using HeLa cells and *in vivo* assays such as accumulation assays and killing assays using *Caenorhabditis elegans*. The virulence assays for these two mutants are under progress and the results will be presented in BacPath 15 conference. The clear understanding of these bacteriophage genes in the survival of *S. flexneri* in the human host can pave the way to the identification of potential attenuation targets and vaccine candidate antigens.

**210**

## Identification and mobilization of the brasiliquinone biosynthesis gene locus from a human-pathogenic isolate of *Nocardia brasiliensis*

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The brasiliquinones are a family of benz[*a*]anthraquinone antibiotics with activity against Gram-positive and acid-fast bacteria. Despite their discovery over two decades ago, the biosynthetic locus responsible for production of the brasiliquinones has remained hidden. Here, we used a combined genomic and genetic approach to identify and clone the brasiliquinone biosynthetic gene cluster from a human-pathogenic isolate of *Nocardia brasiliensis* using transformation-associated recombination (TAR) in yeast. We show that this locus is conserved in other human-pathogenic *N. brasiliensis* and *Nocardia vulneris* genomes and that it is silent in these organisms, but activated upon heterologous expression in a *Streptomyces* host. This work provides the basis for linking quiescent biosynthetic loci to their cognate metabolites in the genus *Nocardia* and forms the first step in rational engineering attempts on the brasiliquinone pathway.

**211**

## The AceI multidrug transporter plays an important role in virulence of the nosocomial pathogen *Acinetobacter baumannii*

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Publish consent withheld

**212**

## Characterising the function of the M-related protein in *Streptococcus pyogenes* virulence

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Group A *Streptococcus* (GAS) is a Gram-positive pathogen ranked as the ninth leading infectious cause of human mortality worldwide by the World Health Organisation (WHO). There is no vaccine available; however vaccine development to date has focused significantly on the highly abundant GAS cell surface M protein. This has paralleled the extensive characterisation of the molecular mechanisms of M protein mediated GAS pathogenesis. However, M protein derived vaccine development has been hindered due to diversity of the M protein between GAS isolates. The M protein is located in the multiple gene activator regulon of GAS adjacent to the M related protein (Mrp) and the M like protein (Enn). Mrp is a GAS surface protein encoded in the genome of approximately 80% of GAS strains. There is evidence to suggest that Mrp plays a multi-faceted role in pathogenesis, contributing to immune evasion, adherence to host cells, and biofilm formation. Mrp has also been identified as a potential vaccine candidate, and incorporation of Mrp into a multivalent vaccine may provide additional coverage in regions of high M diversity. However the molecular mechanisms underpinning the role of Mrp in GAS pathogenesis need to be further investigated, with the consideration of Mrp diversity and structure. Using surface plasmon resonance, we have characterised the ability of phylogenetically diverse Mrp proteins to bind host ligands including fibrinogen and IgG. Understanding which host proteins interact with Mrp on the GAS cell surface, and the degree of binding diversity within the Mrp family will further the understanding of Mrp function, enhancing our understanding of the molecular mechanisms underlying GAS disease.

**213**

## Regulatory RNA contributing to *Listeria monocytogenes* cold adaptation

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*Listeria monocytogenes* is a facultative intracellular bacterial pathogen that has the capacity to grow at refrigeration temperatures. This trait is one of the major factors affecting control of this pathogen in the food chain. We have performed RNASeq and proteomic analyses during growth of this pathogen at 4ºC. *L. monocytogenes* up-regulates a few surface proteins as well as a set of small RNA (sRNA) in the cold. Over-expression of one of these sRNA promoted growth of the pathogen at 4ºC while decreased the growth rate at the host temperature (37ºC). We also obtained evidence for the production at 4ºC of several virulence proteins that *L. monocytogenes* uses to colonize the host. Our data therefore indicate that cold adaptation in *L. monocytogenes* is intimately interconnected with the virulence program activated following host encounter and that RNA regulatory molecules contribute to this interplay.

**214**

## Control of Pseudomonas aeruginosainfections using a biofilm targeting, nitric oxide releasing prodrug

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*Pseudomonas aeruginosa*is associated with the establishment of chronic, antibiotic recalcitrant infections and is considered to be a pathogen of priority. By understanding the genetic and physiological factors that drive biofilm formation, we have discovered that biofilms can be induced to disperse through the addition of nitric oxide (NO), which acts as a non-toxic signal. NO can disperse biofilms of many different bacteria and is effective against multispecies biofilms. We have shown that NO acts through modulation of the secondary messenger, c-di-GMP to control biofilm

formation and that cells respond by producing the NO scavenger Fhp to limit dispersal. Furthermore, high iron levels can antagonise the activity of NO. Dispersed cells become sensitive to antibiotic treatment as well as other biocides, suggesting that NO can act in concert with other bioactive compounds to help eliminate this pathogen. We have shown that NO treatment of CF patients in the clinic can reduce biofilms of *P. aeruginosa* in the sputum of CF patients. To enhance the drug-like features, we have developed NO prodrugs that release NO specifically in the presence of biofilms. The compounds have desirable pharmacokinetic properties, safety profiles and are effective in mouse infection models for biofilms at concentrations similar to or lower than current therapeutics. Based on these data, we suggest that modulation of the biofilm life-cycle, i.e. by inducing dispersal using NO, is a viable strategy for the treatment of chronic infections caused by *P. aeruginosa*.

**215**

## Molecular evolution of imipenem-resistance in clinical isolates of *Klebsiella* spp

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Multidrug resistance in *Klebsiella* species in response to selective pressure and the extent to which it may be reversible is not yet fully understood. Since carbapenem-resistant Enterobacteriaceae (CRE) are considered as a public health threat by the World Health Organization (WHO) and the Centers for Disease Control and prevention (CDC), more studies need to be done to comprehend the evolution of their antibiotic resistance mechanisms to determine if it is possible to reverse this trend.

*K. quasipneumoniae* FK688 is a clinical isolate that was shown to be resistant to imipenem and have lost functional porin expression, one of the frequently occurring mechanisms of clinical multidrug resistance, such as inactivation of porin pump OmpK36 in *K. pneumoniae*. Since genome sequencing indicated that the *ompK36* gene in FK688 was mutated, and potentially responsible for the increased resistance to imipenem, it is an interesting model to study the evolution process.

To study the evolution of imipenem resistance in FK688, the *ompK36* gene in this strain is reverted to the wildtype "*ompK+"* genotype, where porin functionality is recovered, and therefore imipenem sensitivity is restored. The aim of this study is to compare the engineered imipenem-sensitive FK688 "*ompK+"* to the drug-resistant "*ompK-"* progenitor FK688 strain using evolution experiments a competitive fitness assay, where the fitness cost of the mutation at different times points will be monitored. Subsequently, whole genome sequence data will be used to evaluate the rates and occurrence of genetic changes occurring within the *ompk36* gene along with other mutations, and how the *"ompK+*" strain evolves in the presence of antibiotic selection pressure

By identifying the evolutionary trajectory of the "*ompK+"* strain, this study will help us better understand how multidrug resistance potentially develops in clinical settings.

**216**

## The Application of Whole Genome Sequencing in the Prediction of Phenotypic Antimicrobial Resistance in Non-typhoidal *Salmonella* Circulating Australia

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*Salmonella* is the second causative agent of foodborne bacterial gastroenteritis in Australia, which also has the highest *Salmonella* notification rate in comparison to other industrialised countries. With the number of cases increasing annually, antimicrobial resistance (AMR) patterns are also emerging. Whole-genome sequencing (WGS) has paved the way for *in-silico* analysis providing improved genomic techniques and surveillance. The objective of this study was to determine whether WGS could replace phenotypic sensitivity testing at the Microbiological Diagnostic Unit Public Health Laboratory (MDU PHL), the national *Salmonella* reference laboratory. The final dataset included 3,656 *Salmonella* isolates that had both genotypic data obtained from WGS and phenotypic data that incorporated agar dilution sensitivities on nine clinically relevant antimicrobials interpreted using Clinical and Laboratory Standards Institute guidelines. Overall, the sensitivity and specificity rates were above 98% with 0.22% (*n*=67/29,785) discrepant combinations primarily due to streptomycin. AMR was observed in 831 isolates of which 473 were multidrug resistant (MDR, resistance to ≥3 antimicrobial classes) expressed by 25 serovars. Genes encoding resistance was highest against ampicillin ($bla_{\text{TEM-1}}$) followed by sulphathiozole (*sul2*), tetracycline (*tet*A), streptomycin (*strA-strB*), cefotaxime ($bla_{\text{CMY-2}}$) and chloramphenicol (*floR*) with an *S*. Kentucky ST198 and *S*. Indiana ST17 isolate displaying resistance to 7/9 antimicrobials. The two prevalent MDR antibiograms were resistance to ampicillin, chloramphenicol, cefotaxime, streptomycin, sulphathiozole and tetracycline (ACCfSSuT) and resistance to ASSuT, and were mainly observed in *S*. 4,[5],12:i:- ST34 isolates with the most common genotype being $bla_{\text{TEM-1}}$, $bla_{\text{CTX-M-55}}$, *catA2*, *floR*, *strA-strB*, *sul2*, *tet(A)* and $bla_{\text{TEM-1}}$, *strA-strB*, *sul2*, *tet(B)*, respectively. For MDR *S.* Enteritidis ST11 isolates, resistance to ampicillin, sulphathiozole and tetracycline with decreased ciprofloxacin susceptibility (ACpISuT) was observed in 17/39 (43.59%) isolates with $bla_{\text{TEM-1}}$, *sul2*, *tet(A)* and *gyrA*[87:D-Y] being the most frequent genotypic pattern. In conclusion, this study demonstrated a high correlation between WGS and phenotypic sensitivity testing and highlighted that WGS can significantly improve AMR surveillance by simultaneously screening for AMR and emerging multidrug resistance patterns as compared to routine phenotypic methods.

**217**

## Using transposon-directed insertion site sequencing to investigate P. multocida pathogenesis

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*Pasteurella multocida* is a Gram-negative coccobacillus that is both a normal commensal and a primary pathogen of a wide range of mammals and birds. *P. multocida* is the causative agent of several different diseases in different animals that collectively have a large economic impact on global agricultural industries. To better understand *P. multocida* pathogenic mechanisms, we optimised Transposon-directed insertion site sequencing (TraDIS) for use in *P. multocida*. TraDIS allows for identification of genomic regions essential for bacterial growth in any condition of interest.

Using the *Himar1* transposon we produced a TraDIS library with 81,927 unique insertions in *P. multocida* strain VP161 (average insertion every 28 bp). We then used this library to screen for essential genes during growth in rich media and growth in chicken serum, and also to select for genes essential for polysaccharide capsule production. Selection in rich medium identified 481 essential genes; 454 (94%) of these had homologs in the database of essential genes. Selection in chicken serum identified 488 essential genes, of which 40 were essential for growth in chicken serum but not rich medium. Selection for non-capsulated mutants via Percoll gradient centrifugation identified genes likely to be required for capsule production. These included previously identified capsule biosynthesis genes and transcriptional regulators (*fis* and *hfq*), as well as numerous genes not previously associated with capsule production. Confirmation of genes identified as essential for each condition is ongoing via directed mutagenesis. These analyses will allow for the design of novel therapeutic strategies to combat *P. multocida* infections.

**218**

## Unraveling the function of the non-essential components of the BAM complex using Transposon Directed Insertion Site Sequencing.

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In Gram-negative bacteria the β-barrel assembly machinery (BAM) folds and inserts proteins into the outer membrane. Without the BAM complex, β-barrels are not inserted, leading to membrane disruption and demise of the cell. Thus, an increase in the understanding of the BAM complex might help the identification of novel antimicrobial targets. The *Escherichia coli* BAM complex consists of 5 subunits: BamA; BamB; BamC; BamD; and BamE. Many studies have highlighted the importance of the essential components, BamA and BamD. However, the functions of the non-essential BAM subunits, BamB, BamC and BamE are unknown. In this study the transposon-directed insertion site sequencing (TraDIS) approach was used to compare insertion frequencies in mutants defective in one of the non-essential genes with the parent strain. Genes with few or no transposon insertions were suggested to be essential while genes with numerous insertions were classed as non-essential.

TraDIS is a high-throughput technique that involves sequencing of the insertion sites of a high density transposon insertion library. Libraries were produced in mutants defective in *bamB, bamC or bamE*. In a Δ*bamB* mutant, 36 genes were found to be essential that were not essential in the parent strain. The corresponding results for *bamC* and *bamE* mutants were 48 and 18, respectively. Preliminary analysis revealed that genes involved in cell division and lipopolysaccharide assembly have increased importance in a Δ*bamB* mutant while many genes of unknown function have increased importance in Δ*bamC* and Δ*bamE* mutants. Thus, the TraDIS approach can be used to yield insights into the biogenesis of the bacterial outer membrane. The data illustrate how the Tradis approach can be used to help identify potential targets for antibacterial drug development.

**219**

## Phenotypic characterisation of the LirAB two-component system of *Mycobacterium tuberculosis*

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Given the enormous health burden of Tuberculosis, great effort has been invested into understanding the molecular mechanisms governing mycobacterial pathogenesis. The ability of *Mycobacterium tuberculosis* to sense and adapt to changes in its environment is key to its long-term survival within its host cell. Two-component systems (2CRs) represent important genetic regulatory elements that enable efficient responses to these environments. We sought to characterise a novel mycobacterial 2CR, LirAB, and understand its control of key virulence and persistence-associated genes within its regulon. Transcriptional analysis demonstrates LirAB responds to several intra-macrophage environments, including changes in pH and iron, and regulates the expression of genes involved in fatty acid metabolism, cell-wall stress and lipid detoxification. Further characterisation of one such LirAB-controlled gene, referred to here as *ppvp*, is associated with host adhesion and enhanced cell death during infection of murine macrophages. Molecular tracking of PPVP-coated beads indicates this protein localises to the nuclear membrane following internalisation. Our work suggests a multi-functional role for the LirAB 2CR and highlights the importance of environmental sensing on the regulation of *M. tuberculosis* infection pathways.

**220**

## Detailed genomic analysis of *Pseudomonas aeruginosa* piliated reference strain PAK

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*Pseudomonas aeruginosa* is a deadly opportunistic pathogen that has been flagged by the World Health Organisation and classed as one of the highly drug-resistant nosocomial "ESKAPE" pathogens. First described in 1971, *P. aeruginosa* strain PAK is a piliated laboratory reference strain, globally used to study *P. aeruginosa* virulence and pathogenesis. Despite this, a closed, high quality whole genome sequence (WGS) does not yet exist. Here, we report the WGS of *P. aeruginosa* PAK. A Pacific Biosciences (PacBio) RS II sequencing platform was used for WGS, generating 6,395,872 base pairs in one single chromosomal contig with over 65x coverage. The PAK genome contains 5763 CDS and 97 RNA, with an average GC content of 66.44%. *De novo* annotation was performed using PROKKA[1] followed by a secondary annotation transfer from reference strain PAO1 with RATT[2]. Further manual annotation was performed by searching for resistance genes using ResFinder 2.0[3], and 5 acquired resistance genes were identified, all of which are also in PAO1. PHAST[4] was used to identify 2 potential bacteriophages and IslandViewer 4[5] identified 8 genomic islands. The type IV pilus genes pilA-F, pilG-K and chA-C, pilM-Q, pilR-Z, and fimL, S-V were identified. The 3 Type VI secretion system (T6SS) clusters H1, H2 and H3 were also identified. Comparisons of PAK with reference strain PAO1 revealed 44,127 SNPs between the strains and 5421 orthologous genes, as defined by OrthoFinder[6] using default settings. We identified a large inversion of 4.19 Mbp between the strains and the possible insertion site: two sets of rRNA genes of 5847 bp with >99% sequence identity flank the inversion in a complementary orientation. The complete genome sequence of *P. aeruginosa* PAK will advance future understanding of this pathogen, whilst also allowing decades of research on this strain to be placed into genomic context.

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**221**

## Flagellin from uropathogenic *E. coli* induces IL-10 during acute urinary tract infection

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Innate immunity to urinary tract infection (UTI) caused by uropathogenic *Escherichia coli* (UPEC) engages interleukin-10 (IL-10) early to regulate inflammation and promote the control of bladder infection. The mechanism of engagement of innate immunity by UPEC that leads to elicitation of IL-10 in the bladder is unknown. Here, we identify the major UPEC flagella filament, FliC as a key bacterial component sensed by the bladder innate immune system and responsible for the induction of IL-10 synthesis. IL-10 responses of human bladder epithelial cell-monocyte co-cultures as well as mouse bladder were shown to be triggered by flagella of three major UPEC representative strains CFT073, UTI89 and EC958. FliC purified to homogeneity induced IL-10 and other functionally related cytokines, including IL-6 but not IL-12. Characterization of the genome-wide innate immunological context of FliC-induced IL-10 in the bladder using RNA-sequencing identified a 1400-gene network of transcriptional and antibacterial defences induced by FliC. Of the FliC-responsive bladder transcriptome, the changes in expression of *il10* and 808 additional genes were dependent on Toll-like receptor 5 (TLR5), according to comparative analysis of TLR5-deficient mice. Exploration of the potential of FliC and its associated innate immune signature in bladder to protect against UTI revealed significant benefit for the control of infection in mice that received FliC prophylactically or therapeutically after transurethral UPEC infection. We conclude that detection of FliC through TLR5 triggers rapid IL-10 synthesis in the bladder, and FliC represents a potential immune modulator for the treatment or prevention of UTI.

**222**

## Characterisation of the binding affinity of a number of outer-membrane proteins of the pathogen *Haemophilus influenzae* biogroup *aegyptius*, the cause of the lethal febrile disease Brazilian Purpuric Fever

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*Haemophilus influenzae* biogroup *aegyptius* is a common cause of purulent conjunctivitis. It is also responsible for severe invasive disease with a similar presentation to meningococcal septicaemia, characterised by a rapid disease onset. First identified in the state of São Paulo, Brazil, the

disease was referred to as Brazilian purpuric fever (BPF). We aim to investigate how these strains of *H. aegyptius* became so virulent, and what might separate BPF strains of *H. aegyptius* from non BPF strains.

*H. aegyptius* encodes a number of virulence factors with homology to known adhesins in non-typeable *Haemophilus influenzae* (NTHi). *H. aegyptius* encodes two high molecular weight (HMW) adhesins, Hae HMW1 and Hae HMW2, which are related to, yet distinct from, the phase variable HMW1/2 adhesins which have previously been shown to play an important role in colonisation by NTHi. We have previously demonstrated that the HMW1 and HMW2 adhesins of NTHi bind to human-specific glycans found throughout the respiratory tract. *H. aegyptius* also encodes multiple variable trimeric autotransporter (TAA) proteins, named the Tab/Tah proteins. Many TAAs function as adhesins, and a related TAA in NTHi, Hia, is required for host colonisation, and like the HMW proteins, also binds host-specific glycans. We hypothesise that these adhesins of *H. aegyptius,* Hae HMW1/2 and the TAAs Hae Tab and Hae Tah, bind host specific glycans that are distinct from those bound by homologues in NTHi. We cloned and over-expressed Hae HMW1/2 and Hae Tab/Tah proteins in *E. coli* BL21, and utilized a glycobioanalytical approach to determine the binding affinities of these proteins. Each adhesin was screened using the Institute for Glycomics glycan array, and specific protein-glycan interactions characterised using surface-plasmon resonance. By characterising the glycan binding profile of these adhesins, we are better able to understand the factors behind the unusual virulence of a subset of *H. aegyptius* strains that has so far not been elucidated since the first emergence of BPF over thirty years ago.

**224**

## High-resolution transcriptomes of a methicillin-resistant Staphylococcus aureus clinical strain during antibiotic responses

**Sylvania Wu[1], Jai Tree[1]**

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*Staphylococcus aureus* is an opportunistic pathogen that may cause severe disease including sepsis, endocarditis, and pneumonia. Methicillin-resistant *S. aureus* (MRSA) is emerging as a major cause of hospital-acquired infections, and these isolates often acquire resistance to multiple antibiotics limiting treatment options. Current last-line options for treatment of MRSA include vancomycin, linezolid, and tigecycline however, *S. aureus* strains with resistance to last-line antibiotics have emerged. *S. aureus* with vancomycin intermediate susceptibility (VISA), defined as a vancomycin minimal inhibitory concentration between 4 and 8 μg/mL, is a major cause of vancomycin treatment failure. The molecular basis of vancomycin-intermediate susceptibility is not fully understood although phenotypic features such as increased cell wall thickness have been linked to VISA strains,.

Bacteria respond to many acute external stresses through regulatory non-coding RNAs. *Cis*-regulating ncRNAs control transcription and translation of genes within the same locus and include attenuators that regulate transcription by promoting early termination in response to specific ligands or ribosomal pausing. Previously, the Term-seq method was developed to detect RNA termination sites and has been used to identify antibiotic responsive attentuators in *Bacillus subtilis* (Dar et al.,2016).

In the present study, we have used dRNA-seq to identify RNA 5' ends and Term-seq to identify RNA 3' ends, providing a map of transcriptome architecture into *S. aureus*. We have additionally mapped RNA 3' ends in cultures treated sub-lethal dosages of vancomycin, linezolid, or tigecycline for 10 minutes. A computational pipeline was written to predict antibiotic-responsive attenuators using a combination of the Term-seq and dRNA-seq data. This pipeline will be used to find transcripts with condition-dependent termination, and regulated termination sites in 5'-untranslated (UTR) regions of genes. Collectively these data provide a high-resolution map of the transcriptome in MRSA and will identify novel antibiotic-responsive RNA structures in this important human pathogen.

**225**

## Small RNA networks and vancomycin tolerance in *Staphylococcus aureus*

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*Staphylococcus aureus* is a major opportunistic human pathogen and a leading cause of bacteraemia, infective endocarditis, and medical device-related infections. The advent and use of antibiotics made it possible to treat *S. aureus* infections effectively. However, the acquisition of antibiotic resistance has led to the emergence of drug resistant clones of *S. aureus*, termed multidrug-resistant *S. aureus* (MRSA). MRSA isolates that exhibit intermediate resistance to vancomycin, a last line antibiotic used to treat multi-drug resistant Gram-positive pathogens, are increasingly detected worldwide. Vancomycin-intermediate *S. aureus* (VISA) appear to arise from the acquisition of a disparate series of point mutations that lead to physiological changes including cell wall thickening and decreased autolysis.

Transcriptional profiling has revealed that changes in small RNA expression in *S. aureus* are correlated with antibiotic treatment and may contribute to the VISA phenotype. However, the functions of the hundreds of small RNAs in *S. aureus* are still poorly understood. The endoribonuclease RNase III processes sRNA-mRNA duplexes and we have used this protein as a scaffold to capture the sRNA-mRNA interaction network after antibiotic treatment using RNase-CLASH (cross-linking, ligation and sequencing of hybrids). To understand how these vancomycin-responsive sRNA interactions drive changes in the proteome, we will use Ribo-seq to map the precise position of translating ribosomes (ribosome protected fragments, RPFs) after vancomycin stress. Analysis of RPFs will elucidate how mRNA translation rates, protein abundance and ribosome occupancy are affected by vancomycin-induced sRNAs. Together, the above analyses will provide insight into the post-transcriptional and translational changes induced by sRNA-responsive networks in *S. aureus* to generate the VISA phenotype and adapt to antibiotic stress.

**226**

## How isscarce and essential manganese acquired by *Streptococcus Pneumoniae* at the host-pathogen interface?

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*Streptococcus pneumoniae*(the pneumococcus) is a bacterial pathogen of global significance, responsible for more than 1 million deaths per year. Essential to the pathogenicity of *S. pneumoniae*is the efficacious acquisition of transition metal ions at the host-pathogen interface. Accordingly, the mechanisms that facilitate metal ion uptake are potential antimicrobial targets. The type II ATP binding cassette (ABC) transporter PsaBCA is the sole manganese ($Mn^{2+}$) acquisition pathway in the pneumococcus and is essential for *in vivo* virulence. The ABC permease complex comprises a homodimer of heterodimers ($PsaB_2C_2$), to which $Mn^{2+}$is delivered by the extra-cytoplasmic protein PsaA. Here, we investigated the structure and activity of PsaBC, and its interaction with PsaA. The structure of PsaBC was determined by X-ray crystallography and refined to 2.85 Å resolution. Assisted by molecular dynamics simulations of membrane-embedded PsaBC, analysis of the structure identified residues potentially involved in the $Mn^{2+}$translocation mechanism, which include a putative metal ion binding site, translocation gates and docking residues. Comparative sequence analysis revealed that similar residues recur in many transition metal ion ABC permeases and indicates an essential function. The roles of these residues were investigated by alanine substitution into the psaC gene in *S. pneumoniae.* The competence of these mutant strains was assessed by growth in low $Mn^{2+}$media and the accumulation of metal ions was measured by inductively coupled plasma-mass spectrometry (ICP-MS). It was found that residues participating in putative binding sites in the internal channel and at the PsaA/PsaC interface are indispensable for accumulation of $Mn^{2+}$and growth. The crucial role of these conserved residues strongly suggests that PsaBC interacts directly with the metal ion in order to facilitate directional transport through the transmembrane domains; indicating a novel molecular mechanism for solute import distinct from other Type II ABC permeases. This finding resolves a fundamental question as to how bacterial cells import $Mn^{2+}$ions and provides structural and functional insights essential for future antimicrobial design.

**227**

## Evolution of improved secretion, function and fitness is driving the emergence of new alleles of the NDM-1 drug resistance gene.

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The New Delhi metallo-b-lactamase (NDM-1) mediates resistance to b-lactam antibiotics. NDM-1 was created by a fusion between sequences encoding the first six amino acids of cytoplasm-localised aminoglycosidase, AphA6, and a periplasmic metallo-b -lactamase. We show that NDM-1 has an atypical signal peptide and is ineffficiently secreted. Using mass spectrometry we show that NDM-1 is cleaved by *E. coli* signal peptidase I between L21/M22 of the precursor protein. We find no evidence that NDM-1 is a lipoprotein, as has been reported elsewhere. Two new $bla_{NDM-1}$ alleles that have polymorphisms in the signal peptide; NDM-1(P9R), a proline to arginine substitution, and NDM-2, a proline to alanine substitution (P28A). We show that both the P9R and P28A substitutions improve secretion compared to NDM-1. Mass spectrometry analysis of these purified NDM proteins showed that the P28A mutation in NDM-2 creates new signal peptide cleavage sites at positions 27 and 28. NDM-1(P9R) and NDM-2 exhibit improved secretion, increased resistance to some antibiotics and expression of NDM-2 improves the fitness of *E. coli* compared to NDM-1, in the absence of antibiotic selection. Further optimization of the secretion efficiency of this metallo-b-lactamase may give rise to new alleles with increased resistance and pathogens with increased fitness.

**228**

## Class 1 integrons and antibiotic resistance genes in wild and domestic horses.

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The equine hindgut is a potential source of antimicrobial resistance genes (ARGs) that impact both human and animal health. In this study, we screened equine faecal specimens for the presence of class 1 integrons and associated ARGs. We hypothesised that domestic horses with greater human exposure and veterinary intervention would contain higher levels of ARGs and associated mobile genetic elements such as integrons. Faecal samples were collected from horses on a farm (n=11) and from wild brumbies (n=11). Antibiotic-resistant bacteria were isolated by dilution-plating onto a range of antimicrobials, then colonies were screened for the class 1 integron gene *inti1*. *IntI1* genes, associated gene cassettes and the 16S rDNA of PCR-positive bacteria were sequenced.

Fourteen isolates (10 Gram positives, 4 Gram negatives) from antibiotic agar plates contained class 1 integrons. These were identified by 16S sequencing as *Kocuria palustris, Arthrobacter citreus, Arthrobacter luteolus, Microbacterium esteraromaticum, Micrococcus terreus, Microbacterium aurum, Rhodococcus coprophilus, Escherichia hermanii, Leclercia adecarboxylata*, *Pantoea sp.,* and *Pseudomonas sp.* The *intI1*-positive isolates were obtained on streptomycin, sulfamethoxazole, tetracycline, and trimethoprim plates; they all had conserved 3' sections (*qacE*delta1-*sul1*), and they yielded six distinct gene cassette array types: *aadA2*; *aadA9*; *dfrA5*; *dfrA16*; *dfrA1-aadA6*, or no cassettes.

The prevalence of Gram-positive bacteria among the class 1 integron positive isolates was unexpected. Further analysis of these isolates by complete genome sequencing is underway.

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