The President of the Australian Society for Microbiology and the Local Organising Committee welcome you to the ASM’s 43rd Annual Scientific Meeting and Trade Exhibition. Australia’s investment in microbiology over the past century has paid handsome dividends. From celebrated medical discoveries to the every day exploitation of domesticated microbes, the microbiology community in Australia, represented collectively by the Australian Society for Microbiology, have much to be proud of.

This year we have removed the traditional divisions recognized by the society (medical/veterinary, virology, environmental, molecular) to present a more fluid and integrated scientific program. The conference theme - One Microbiology - celebrates a passion for microbiology in all environments regardless of purpose and recognizes the profound importance of microbiology and microbiologists to the future of human and environmental health on Earth.

The conference is preceded by the 2nd Annual ASM Educon conference for microbiology educators at ANU’s University House 11-12 July 2015, a feast of workshops at QT Canberra 12 July 2015, and the Sunday afternoon Public Lecture – Guts to Great Oceans (Jansson, Manefield, Giovannoni) – co-hosted by the Australian Academy of Sciences in the Shine Dome 12 July 2015 with a presence from Questacon. Formal proceedings will commence on Sunday the 12th of July with the Bazeley Oration delivered by acclaimed virologist Yoshihiro Kawaoka specializing in influenza and Ebola viruses, followed by a Welcome Reception with Trade Sponsors at QT Canberra.

The scientific program runs Monday to Wednesday (13-15 July 2015) at QT Canberra showcasing some of the most influential local and international microbiologists in the world. Invited international plenary speakers include Stefan Schwarz (Germany), Chantal Abergel (France), Stephen Giovannoni (USA), Judith Berman (Israel) and Jorge Galan (USA). Acclaimed microbial ecologist of the human gastrointestinal tract Janet Jansson (USA) will deliver the Rubbo Oration on Tuesday evening followed by a formal Rubbo Supper and late night revelry in QT Canberra’s draw card bar Lucky’s. The Chief Medical Officer of Australia Chris Baggoley will deliver the Snowdon Oration on Wednesday morning. The Annual General Meeting of the Society will take place over lunch on the Tuesday. The Australasian Mycological Society will join the meeting for the final day on Wednesday 15th July 2015 flowing into an independent venue on Thursday the 16th July 2015.

Students and Early Career Researchers are invited to a host of career advancement events including the Nancy Millis Student Mentoring Breakfast and Lunch, the ECR Mentoring Lunch (focus on funding) and an evening of brewing, old school social networking and unwinding at the awesome Bentspoke Brewery, Canberra. Three top tier poster and trade sessions are woven into the program displaying as much as possible of the talent and tech that we couldn’t fit on stage.

Mike Manefield
LOC Chair, ASM2015

Jon Iredell
President, ASM

ASMS National Office
9/397 Smith Street, Fitzroy VIC 3065
1300 656 423
Kara Taglieri
Maree Overall
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The Organising Committee for the Australian Society for Microbiology Annual Scientific Meeting and Exhibition acknowledges with gratitude the generous support received from the following sponsors and exhibitors.

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Genetic Signatures

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In Vitro Technologies

Iaftech

Roche

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<td>CAPSIG Workshop</td>
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<td>Janet Jansson, Stephen Giovannoni and Mike Manefield</td>
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<td>Location: Academy of Science, Shine Dome</td>
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<td>07:15</td>
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<tr>
<td>08:30</td>
<td>Plenary 1 - Stefan Schwarz: New antimicrobial resistance genes in MRSA and other staphylococci of animal origin</td>
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<td>Plenary 3 - Chantal Abergel: The exciting universe of giant viruses</td>
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<td>09:15-10:00</td>
<td>Plenary 4 - Stephen Giovannoni: Predicting long-term impacts of ocean desertification on microbial plankton communities</td>
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<td>10:00-10:30</td>
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| 10:30-12:10 | Epidemiology Chair: Andrew Gordon
Stefan Schwarz
Grant Hill-Cawthorne
Sebastian Van Hal
Rosemarie Sadsad
Ruiting Lan
David Cleary

Host Pathogen Interactions I Chair: Michael Frese
Barry Slobedman
Markus Hofer
Maria Liaskos
Jamie Triccas
Jaelle Brealey
Bishara Marzook

Molecular Genetics & Evolution Chair: Dena Lyras
Miles Davenport
Eddie Holmes
Giel Van Dooren
Slade Jensen
Sanja Trifkovic
Hannah Read |
| 12:10-13:30 | Lunch                                                                           |
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Lucy Furfaro
Amelia Hynen
Hayden Thain
Sushama Telwatte
Christopher Atkinson
Agathe Calmant |
| 15:00-15:30 | Afternoon tea                                                                    |
| 15:30-17:10 | Emerging Pathogens Chair: Grant Hill-Cawthorne
Greg Moseley
Jason Roberts
Cameron Webb
Greg James
Michael Nissan

Molecular Pathogenesis Chair: Ruiting Lan
Jorge Galan
Victoria Korolik
Charlene Kahler
Peter Waterhouse
Eleanor Latomanski
Jai Tree

Animal Diseases Chair: Gary Muscatello
Peter Walker
Michael Ward
Paul Hick
Lee Skerratt
Carla Giles
Lechelle van Breda |
<p>| 17:10-18:30 | Posters B                                                                        |
| 18:30-19:30 | Rubbo Oration - Janet Jansson: Multi-omics of the human gut microbiome           |
| 19:30      | Rubbo Dinner                                                                     |</p>
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<td>Antimicrobials &amp; Resistance</td>
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<td></td>
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<td>Rowena Martin</td>
<td>Rowena Martin</td>
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<td>Chair: Alex Idnurm</td>
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<td>Melissa Brown</td>
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<td>Dale Domey-Howes</td>
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<td>Emma Peel</td>
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<td>Chair: Kim Plummer</td>
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<td>William Klare</td>
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<td>Omics and Systems Biology</td>
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<td>Amy Kennison</td>
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<td>Vivek Pande</td>
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Welcome to Canberra for One Microbiology, the 2015 Scientific Meeting of the Australian Society for Microbiology.

This year we have brought in some changes to our meeting. We recognize that microbiology is an increasingly interwoven and interdependent science, with microbial ecology now seen as an important component of medical and industrial microbiology, agricultural pathogens stepping into the clinical area, viruses playing a role ecosystem health as well as disease, and molecular biology and genetics underpinning almost all of our research. In line with this and our One Microbiology theme, we are mixing things up so that symposia no longer run along traditional Division lines, allowing us to explore today’s most significant issues from every angle of microbiology. At the same time we’ve been careful to maintain a balance across the Division themes, so you will get to hear the latest progress in your specialty area, while also getting to look at this from different and possibly new perspectives.

We have an outstanding line-up of presenters, including plenary speakers joining us from Europe, the United States and Israel, Bazeley and Snowdon orators, winners of the Fenner, Becton-Dickinson and Lyn Gilbert awards, and a broad selection of symposium speakers showcasing the best of microbiology research conducted in Australia today. I’d like to thank the ASM Division Chairs, Tim Newsome, Nick Coleman, Mitchell Brown and Slade Jensen for putting together the scientific program, and LOC chair Mike Manefield and all members of the LOC for their contributions to organising our meeting.

A special welcome to our younger members: the students and the early career researchers. You are the future of Australian microbiology and we want to ensure you stay with us! To support this we have organized special social and mentoring functions that are aimed at helping you as you begin your microbiology careers. We hope you will feel welcome, happy to be part of our Society, and excited to continue your involvement in the exciting and expanding world of microbiology.

It’ll be cold outside in Canberra, so get comfortable in the stylish QT hotel, network with your peers in its bars, enjoy your coffee while browsing posters and trade exhibits, catch up with old friends and colleagues, make new ones, sit back and absorb some of the best microbiology you will hear all year, and come away inspired and ready for more work at the microbial coal face!

Dee Carter
Chair, Scientific Program Committee, ASM2015

ORGANISING COMMITTEE

<table>
<thead>
<tr>
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<th>Scientific Program Chair</th>
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<tr>
<td>Mike Manefield</td>
<td>Dee Carter</td>
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Committee Members

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<tr>
<td>Ricardo Alfon Guzman</td>
<td>Slade Jensen</td>
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<td>Christel Armstrong</td>
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<td>Mitchell Brown</td>
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<td>Naresh Verma</td>
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<td>Thomas Jeffries</td>
<td>Charlotte Webster</td>
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PLENARY SPEAKERS

Chantal Abergel
The structural team is currently focusing on large DNA viruses such as Mimivirus and PBCV. A comprehensive structural and functional study of selected genes products was initiated, as they may hold important clues about the origin of DNA viruses and on the mechanisms used by viruses to hijack the host cell to its own benefit. We first focused on genes seen for the first time in a virus such as enzymes involved in translation and DNA metabolism. We are now interested in genes conserved in large DNA viruses. Analyzing them is a great opportunity to discover new “entry” points (molecular switches) in the control of cell death (apoptosis), cell division (DNA replication), or bacterial infection (phagocytosis, cell trafficking). This in turn might lead to innovative therapeutic approaches. We produce our protein targets using a standard protocol based on incomplete factorial design (Abergel et al., 2003, J Struct Funct Genomics; 4:141-57). Activity measurement is performed using spectroscopic techniques every time possible or in collaboration with specialized laboratories. For each project we try to solve the structure of the protein of interest alone and in complex with predicted ligands or interactors.

Chris Baggoley
Professor Chris Baggoley is Chief Medical Officer for the Australian Government and is the principal medical adviser to the Minister and the Department of Health. He also holds direct responsibility for the Department of Health’s Office of Health Protection. Prior to his appointment Professor Baggoley was the Chief Executive of the Australian Commission on Safety and Quality in Health Care. He was a former Chief Medical Officer with the South Australian Department of Health. His clinical career has been in emergency medicine. Professor Baggoley was the President of the Australasian College for Emergency Medicine, Chair of the national Committee of Presidents of Medical Colleges and Chair of the Board of the National Institute of Clinical Studies and his previous medical positions include Professor-Director of Emergency Medicine at the University of Adelaide and Royal Adelaide Hospital; Director of Emergency Medicine at Flinders Medical Centre; the inaugural Director of Emergency Services at the Ashford Community Hospital. In addition to his Flinders University Degree in Medicine, Professor Baggoley holds a Flinders University Bachelor in Social Administration and an Honours degree in Veterinary Science from the University of Melbourne. In the Queen's Birthday honours for 2013, Professor Baggoley was made an Officer of the Order of Australia (AO).

Judith Berman
Professor Judith Berman heads a research laboratory in the George S. Wise Faculty of Life Sciences, Tel Aviv University since October 2012 and was previously Distinguished McKnight University Professor in the College of Biological Sciences at the University of Minnesota. Judith received her B.Sc. degree in Plant Pathology at Cornell University and her Ph.D. degree in the field of Biochemistry at the Weizmann Institute of Science. As a post-doctoral fellow at Cornell University she identified the first telomere binding protein, Rap1, in budding yeast. Her lab uses genetics, genomics, cell biology, biochemistry, microbiology and experimental evolution approaches to study the mechanisms underlying the rapid appearance of phenotypes such as drug resistance in Candida albicans, the most prevalent human fungal pathogen. Judith’s lab discovered that segmental and whole chromosome aneuploidies are highly associated with drug resistance and that shifts in whole genome ploidy occur in vitro, in mammalian model systems as well as in clinical isolates. Her lab focuses on how these changes in whole genome and individual chromosome ploidy arise, how they confer selective advantage to cells that contain them, and how to avoid their appearance in order to reduce the prevalence of antifungal drug resistance.
**Jorge Galan**

Jorge Enrique Galán is an Argentine/United States microbiologist who won the 2011 Robert Koch Prize. His research concerned bacterial pathogenesis and he is the Lucille P. Markey Professor of Microbial Pathogenesis at Yale University. Our laboratory studies the pathogenesis of two intestinal pathogens, *Salmonella enterica* and *Campylobacter jejuni*. Combined, these two pathogens account for the majority of cases of infectious diarrhea worldwide leading to an estimated 2,000,000 deaths. We take a multidisciplinary approach in our studies involving bacterial genetics, biochemistry, cell biology, immunology as well as structural biology. As a result, we are beginning to define not only the molecular details of the host pathogen interactions but also the atomic interphase between these pathogens and the host.

**Stephen Giovannoni**

Steven Giovannoni has a PhD from the University of Oregon and currently runs the Marine Microbial Ecology, Physiology and Genomics lab in the Department of Microbiology at Oregon State University, where he also teaches several courses. His research interests include genome evolution and metabolism of the marine bacterium SAR11, the marine carbon cycle, high throughput microbial culturing, proteomics, and metabolomics. The goal of Dr. Giovannoni’s research is to understand how dominant marine bacteria function in global biogeochemical cycles. His research group made the strategic decision to focus on pure cultures of marine bacteria that can be studied in a controlled laboratory setting, and uses metagenomics and oceanographic field work to link laboratory discoveries to natural processes. His work with the Sargasso Sea Microbial Observatory allows him to collaborate with oceanographer Dr. Craig Carlson, as well as Rachel Parsons at BIOS, in a long-term NSF-sponsored project that takes an ecological perspective to understanding the role of microorganisms in the oceanic carbon cycle. The study site in the Western Sargasso Sea (a.k.a. BATS, the Bermuda Atlantic Time-series Study) is an example of an oligotrophic ocean gyre. Seventy percent of the oceans are gyres, regions of clear water and low productivity. At this study site, the research group uses a variety of experimental approaches, including metagenomics, metaproteomics, fluorescence in situ hybridization and chemical measurements, to understand how microbial communities recycle organic matter.

**Janet Jansson**

The overarching aim of my research is to gain a better understanding of key functions carried out by microorganisms in complex microbial communities, such as those residing in soil, sediment and the human gut, by employing state-of-the-art and novel “omics” approaches. These tools are implemented with bioinformatics and statistics using super computing facilities to tackle increasingly large and complex meta-omic datasets. One specific research area that we are addressing in my group is to use omics to better understand the role of soil microorganisms in cycling of carbon and how these functions are perturbed in the face of a changing climate. For example, we are aiming to understand how changes in precipitation will impact the cycling of stored soil carbon in prairie soils and how a warming climate will impact microbial decomposition of carbon stored in permafrost. Another application of omics in my group is to explore high salt environments for novel enzymes for lignocellulose degradation for biofuel applications. We are also using multi-omics approaches to study the impact of the Deepwater Horizon oil spill on indigenous microbial communities in the Gulf of Mexico. Additionally, we have a major research focus on the human microbiome and are studying the impact of antibiotics, diet and disease on gut microbial community composition and function. Examples of our human microbiome research include use of omics to understand how the gut microbiota composition and function is impacted by resistant starch in the diet and by Crohn’s disease.
Yoshihiro Kawaoka

Dr. Yoshihiro Kawaoka was educated in Japan, receiving his DVM in 1978 and his PhD in 1983 from Hokkaido University. Early in his career, he identified a critical determinant for high pathogenicity of avian influenza viruses; this information is now used by the USDA and the Office International des Epizooties (World Organisation for Animal Health, OIE) as a criterion for rapidly identifying lethal and non-lethal avian influenza viruses. Dr. Kawaoka also established the technique of reverse genetics, which allows the generation of ‘designer’ influenza viruses. This technology – coupled with his findings regarding the attenuation of deadly influenza viruses – has been used to develop candidate H5N1 influenza virus vaccines, which have proven efficacious in clinical trials. Reverse genetics is also utilized to generate live attenuated influenza vaccines (FluMist) that are used worldwide. Dr. Kawaoka has also studied the 1918 Spanish influenza virus, which killed over 40 million people. He discovered that infection with this virus caused an abnormal immune response. His findings are used globally by public health agencies as part of the enormous task of influenza pandemic planning.

In addition to his work with influenza virus, Dr. Kawaoka also studies Ebola virus. Because of its extreme virulence, Ebola virus used to be studied only in laboratories designated as biosafety level 4 (BSL4), the highest containment environment possible. This requirement severely hampered the progress of Ebola virus research, as few such facilities exist worldwide. Dr. Kawaoka was the first to establish a pseudotype virus system that allows the analysis of Ebola virus glycoprotein under BSL2 conditions. Dr. Kawaoka has also developed another system that allows the study of the entire Ebola virus replication cycle under non-BSL4 conditions.

In recognition of his achievements, Dr. Kawaoka was awarded the prestigious Robert Koch Award in 2006, and the Medal of Honor (Purple Ribbon) from the Emperor of Japan in 2011 for his innovative research in the field of influenza virology. In 2013, he was elected as a Foreign Associate of the United States National Academy of Sciences.

Stefan Schwarz

Prof. Dr. med. vet. Stefan Schwarz works in the Institute of Farm Animal Genetics of the Friedrich-Loeffler-Institut (= Federal Research Institute for Animal Health) in Neustadt-Mariensee, Germany. His research group ‘Molecular Microbiology and Antimicrobial Resistance’ is involved in both surveillance of antimicrobial resistance and analysis of the molecular genetics of antimicrobial resistance. He also teaches various academic courses at the University of Veterinary Medicine Hannover. Prof. Schwarz is a specialist veterinarian in (a) microbiology, (b) epidemiology, and (c) molecular genetics and gene technology. He has published >300 papers and acts as editor / editorial board member of six international journals.
DELEGATE INFORMATION

WEBSITES
www.theasm.org.au
asmmeeting.theasm.org.au

SECRETARIAT
ASN Events Pty Ltd / ASM National Office
9/397 Smith Street
Fitzroy VIC 3065
Ph: +61 3 8658 9530 | Fax: +61 3 8658 9531

VENUE
QT Canberra
1 London Circuit
Canberra
ACT 2601
Ph: +61 2 6247 6244

ORGANISER’S REGISTRATION DESK
The organiser’s registration desk will be located in the Main Lobby of QT Canberra’s ground floor opposite the hotel registration desk. The desk will be attended at all times during the conference, see hours below. Delegates should collect their satchel and name tag on arrival. A message board will be at the registration desk to help identify ASM2015.

Sunday 12th July: 07:30 – 21:30
Monday 13th July: 07:15 – 21:00
Tuesday 14th July: 07:30 – 19:30
Wednesday 15th July: 07:30 – 17:00

FULL REGISTRATION ENTITLEMENTS:
- Entry to all scientific sessions & the Exhibition Hall
- Access to Conference Material
- Lunch, Morning tea and Afternoon tea
- Attendance at the Welcome Function
- Attendance at the Poster Sessions
- Conference Satchel
- Final program & Abstract Book

DAY REGISTRATION ENTITLEMENTS:
- Entry to all scientific sessions & the exhibition hall on the day of registration
- Lunch, Morning tea & Afternoon tea on the day of your registration
- Conference Satchel
- Final program & Abstract Book

NAME BADGES
You are required to wear their name tags to all scientific and catered sessions.

SMOKING POLICY
Smoking is not permitted in the venue

TAXIS
The hotel reception can arrange taxis as required from the QT Canberra.

TICKETS
Delegates will receive all pre-booked tickets with their name tag. Tickets are required for the Rubbo Dinner. If you would like book this function, please visit the registration desk.

CATERING
Lunch, morning tea and afternoon tea are included in your registration. Refreshments at QT Canberra will be served in the Exhibition Hall.

SPECIAL DIETARY REQUIREMENTS
If you requested a special meal, please make yourself known to the venue staff and advise your name and special request.

INSURANCE
The hosts and organisers are not responsible for personal accidents, any travel costs, or the loss of private property and will not be liable for any claims. Delegates requiring insurance should make their own arrangements.

SOCIAL MEDIA GUIDELINES
During ASM’s annual scientific meeting and conferences, many of our speakers will be presenting exciting novel research that is not yet published. While the society 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, ASM’s social media policy during meetings is as follows:

**We encourage all attendees to interact on social media by:**
- “Liking” ASM on Facebook and sharing your conference experience: https://www.facebook.com/AustralianSocietyForMicrobiology
- Following ASM on Twitter: @AUSSOCMIC, and tweeting about the meeting using #2015ASM
• Joining the ASM LinkedIn group: https://www.linkedin.com/groups/Australian-Society-Microbiology-6605071
• Subscribing to the ASM YouTube channel: http://www.youtube.com/user/AUSSOCMIC

All talks are “bloggable” and “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 ASM scientific meetings. 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.

DISCLAIMER
The hosts, organisers, venue and participating society is not responsible for, or represented by, the opinions expressed by participants in either the sessions or their written abstracts. Responsibility for the literary and scientific content of abstracts accepted for publication remains with the authors and their sponsoring institutions. Acceptance by the Society for publication does not imply any acceptance by the Society of responsibility.

SESSION LOCATIONS
Conference rooms are located on the second level of QT Canberra with Element Room located 1 level. Sunday – Wednesday breaks and lunches take place in the Exhibition Hall.

ORAL PRESENTATIONS
The speaker preparation room is located on the second floor called Studio 4. As the program is running concurrent sessions it is essential that you load your talk to the conference network from the speaker prep room at least 2 hours before your session start time. There will be no provision for people to use their own laptops. The conference presentation software is MS PowerPoint 2007. Those preparing on Macs should save for this output. Any issues should be resolved in the speaker preparation room beforehand. A technician will be present to assist.

POSTER PRESENTATIONS
Delegates with posters can place their poster by finding the appropriate abstract number on the display panels in the exhibition area. The program provides instructions regarding the days which posters should be presented. During the specific poster discussion sessions, presenters must be present talk discuss with delegates. This is also the time when official judging will occur.

MOBILE PHONES AND PAGERS
Please ensure your mobile phone/pager is turned off/or on silent during any session you attend.

OFFICIAL CONFERENCE MOBILE APP
The official ASM2015 mobile app will keep you informed during the meeting. Within the mobile app you can view the entire program, plan your own schedule, view your registration, find abstracts and authors by name and make notes for later reference.

Downloading the ASM2015 Mobile App is easy!
Simply, visit http://asmicro-2015.m.asnevents.com.au and save the page to your Smartphone’s home screen.
SOCIAL FUNCTIONS

Sunday 12 July

Opening Reception – Trade Night - 7:30-9:30pm
It all starts here. Catch up with old friends and meet some new ones over drinks and canapés as we see what our trade partners have to offer.

For those wanting to continue the party the bar at QT will remain open following the event.

Monday 13 July

Student Breakfast - 7:15am-8:15am, Eureka Room
Come meet some other ASM student members and share a casual breakfast provided at the venue

Nancy Millis Student Lunch - 12:30pm-1:50pm, Eureka Room
Join other students in the ‘Eureka room’ for lunch and talk careers with our selected student mentors

ECR Lunch - 12:45pm-1:50pm, Studio 2
Join other Early Career Researchers for a talk about funding and grant opportunities in Australia presented by Fiona Cameron from the Australian Research Council. This will be followed by a general ‘roundtable’ discussion.

Posters A with trade exhibit - 7:00pm-8:30pm
Drinks and canapés will be provided

Student and ECR night at “Bent Spoke Brewery” - 9pm-late
Relax and network at a local craft brewery – some (quality) drinks and nibbles provided

Tuesday 14 July

Posters B with trade exhibit - 5:10pm-6:30pm
Drinks and light refreshments will be provided

Rubbo Dinner - 7:30pm-10:00pm
Australian Microbiology’s ‘night of nights’. A “sit down” 2-course dinner will be provided in the Eureka room.

Rubbo Dinner After Party - 8:00pm-late
Join us for a drink and dance at Lucky’s SpeakEasy.

Wednesday 15 July

Joint ASM/AMS Posters C with trade exhibit - 12:40pm-2:00pm

Australasian Mycological Society (AMS) Social Function - 7:00pm
Dinner at Boffins Restaurant, Australian National University
Please visit the AMS website for details on how to book.
## STUDENT & ECR ACTIVITIES

### Nancy Millis Student Mentoring Breakfast
Date: Monday July 13th 2015  
Time: 07:15 – 08:30 AM  
Venue: Eureka Room

This is an opportunity for the students to network and gain further connections and collaborations within the microbiology field. The event will be structured so that you can meet and discuss aspects of your career with a number of the international and national guests presenting at ASM Canberra 2015.

### Nancy Millis Student Mentoring Lunch
Date: Monday July 13th 2015  
Time: 12:45 – 1:50 PM  
Venue: Eureka Room

A professionally facilitated workshop, where the concepts of the Nancy Millis Mentoring Program will be explained. Student peer groups will be formed and contact details of participants registered and exchanged.

Speaker: Barbara Kameniar

### ECR Mentoring Lunch
Date: Monday July 13th 2015  
Time: 12:20 – 1:50 PM  
Venue: Studio 2

Join other Early Career Researchers for a talk about funding and grant opportunities in Australia presented by Fiona Cameron from the Australian Research Council. This will be followed by a general 'roundtable' discussion.

Speaker: Fiona Cameron

### Student & ECR night at “Bent Spoke Brewery”
Date: Monday July 13th 2015  
Time: 21:00 – late  
Venue: Bent Spoke Brewery - 38 Mort St, Braddon (22 minute walk)

A casual evening for the students to meet, relax and socialise at a local craft brewery. We hope all the students will join us for some nibbles and (quality) drinks and a chance to meet other students from a wide variety of institutes.
Canberra, ACT, Australia

Canberra is Australia’s capital, inland from the country’s southeast coast. Surrounded by forest, farmland and nature reserves, it has earned the nickname of the “Bush Capital.” The city’s focal point is Lake Burley Griffin, filled with sailboats and kayaks. On the lakeshore is the massive, strikingly modern Parliament House, as well as museums including the National Gallery, known for its indigenous art collections.

Currency Exchange
There are many places to exchange currency in the CBD. Banks, travel agencies and dedicated exchange spots are readily available.

Tipping
Melbourne (or anywhere in Australia) does not operate on a tipping culture. You may tip to show your appreciation for good service, but it is not a requirement.

Taxes in Canberra
A Goods and Services Tax of 10% is applied to most goods and services in Australia. All displayed prices for Goods and Services are required to include the GST. If you leave Australia within 30 days of buying goods, you may be exempt from paying GST. You can take advantage of the Tourist Refund Scheme (TRS) to claim back the GST you have paid, and also claim back any Wine Equalisation Tax (WET) you paid. TRS only applies to goods that you can carry as hand luggage, and other conditions do apply.

Climate
Canberra has a relatively dry, continental climate with warm to hot summers and cool to cold winters (at least for Australian standards).

Telephone Information
If calling from a public telephone, the average price for a phone call to another landline within Australia is 50c. Phone cards can be purchased from post offices and newsagents, for local, interstate and international calls. Mobile (cell phone) calls are more expensive. When making international phone calls, the following formula should be used: 0011 + country code + area code (if this has a ‘0’ please omit) + telephone number.

Dining Out
Meal prices range anywhere from AU$10 at local cafes and small restaurants, to AU$200 at world-leading quality restaurants. Please also visit www.urbanspoon.com for an up to date source of restaurant and café reviews and recommendations.

Things to See and Do
Canberra has a multitude of inner city and regional tourist options that are easily accessible from the city. Main attractions include Parliament House, Australian War Memorial, National Museum, National Gallery and the Australian National Botanic Gardens. Other attractions (more suited for children) include the National Zoo and the Australian Institute of Sport.

Transport
Being on a smaller scale city, the transportation methods in Canberra are quite limited. The primary source of public transport inside the city of Canberra is by bus/coach. There are 5 different coach companies that travel interstate to Canberra, 3 interstate trains and multiple airline services that operate through Canberra International Airport.
Thermo Fisher Scientific
Website: www.thermofisher.com.au
Thermo Fisher Scientific is the world leader in serving science, enabling our customers to make the world healthier, cleaner and safer. We are the leading provider of analytical instruments, equipment, reagents and consumables, software and services for research, analysis, discovery and diagnostics.
Supporting our range of Microbiology products is our team of Microbiology experts who have extensive knowledge and expertise in Clinical, Industrial and Academic Microbiology applications. Our dedicated team is focused on supporting your needs, and to the science of Microbiology.

CSIRO
Website: www.csiro.au
CSIRO’s Australian Animal Health Laboratory (AAHL) is a high-containment facility designed to allow scientific research into the most dangerous infectious agents in the world.
AAHL plays a vital role in maintaining the health of Australia’s animals, the international competitiveness of Australian agriculture and trade, the well-being of Australians and the quality of our environment.
As one of only six high-containment animal research centers in the world, AAHL works with national and international human and animal health organisations as part of a global One Health network. It is a national centre of excellence in disease diagnosis, research and policy advice in animal health and is the most advanced laboratory in terms of providing world-class animal health diagnostic services.
CSL

Website: www.csl.com.au

CSL is a global specialty biopharmaceutical company that researches, develops, manufactures and markets biotherapies to treat and prevent serious and rare medical conditions. We produce safe and effective therapies for patients who rely on them for their quality of life and sometimes for life itself, enabling many thousands of people around the world to lead normal healthy lives.

CSL employs over 13,000 staff in more than 27 countries. Our headquarters are in Australia and we have substantial manufacturing operations in the US, Germany, Switzerland and Australia. CSL also has one of the largest plasma collections networks in the world and operates the only influenza vaccine manufacturing facility in the Southern Hemisphere.

Amyl Media

Website: www.amylmedia.com.au

Amyl Media has been Australia’s ONLY Australian owned Media Formulator for over 25 years! From routine micro through to specialty commissioning, we can produce anything from a small ‘tweak’ to a total formulation. Our range includes pre-prepared media, dehydrated media, diluents and plastic/ancillary equipment. Amyl Media prides itself on Accuracy, Integrity and Reliability.

Australian Proteome Analysis Facility

Website: www.proteome.org.au

APAF Ltd - Australian Proteome Analysis Facility, was the birthplace of the term proteomics in 1995 and was the world's first dedicated high throughput proteomics laboratory. APAF has over 18 years of experience in providing proteomic services, and combined with our leading edge infrastructure and expertise we provide total solutions for all your proteomic research needs. APAF's mission is to assist the scientific community address their protein analysis needs.

Beckman Coulter

Website: www.beckmancoulter.com

For over 80 years, Beckman Coulter has partnered with clinical diagnostic laboratories to innovate processes, performance and productivity to help move labs forward.

IRIS Urinalysis Systems are preferred by more laboratories worldwide because they provide advanced and innovative technologies that deliver results integrity and diagnostic accuracy. Iris systems help laboratories to "Work Smarter" by optimizing workflows to enhance service provision and advance healthcare for every person.

Bioline Australia

Website: www.bioline.com/au/

Bioline is a primary manufacturer of high quality Molecular Biology reagents specialising in real time PCR, PCR and cloning. We are part of the Meridian Bioscience group of companies, providing clinical diagnostic and molecular research solutions. We will have details of several new ISOLATE II DNA/RNA Extraction Kits, the Epik range of products for Epigenetics analysis, SensiFAST real-time PCR mixes and MyTaq PCR reagents available at our booth.

bioMerieux

Website: www.biomerieux.com.au

bioMérieux continues its commitment to improving healthcare worldwide through a complete range of in-vitro diagnostic solutions for managing Infectious diseases, Cardiovascular diseases and targeted Cancers. The acquisition of Biofire has strengthened our leadership position in molecular diagnostics. Committed to our strategy of addressing the major challenges for public health, bioMerieux innovate and design the diagnostics of the future, whilst improving laboratory efficiency for the benefit of the patient.

BMG Labtech

Website: www.bmglabtech.com

BMG LABTECH is a leading global developer and manufacturer of innovative, high-quality and reliable microplate reader instrumentation for more than twenty-five years. By focusing on the needs of the scientific community, the company's innovative microplate readers have earned the company the reputation of being a technology leader in the field.
Bruker
Website: www.bruker.com
For over 50 years Bruker's innovative and high performance products have delivered excellence in research, industrial, routine and quality control applications. The MALDI Biotyper extends its best-in-class identification of microorganisms with the addition of high-value functional assays, new software tools, library extensions and productivity tools.

Cell Biosciences
Website: www.cellbiosciences.com.au
As a supporter of the ASM and Australian Microbiologists, Cell Biosciences’ is proud to make available leading international products, supported by superior technical and customer support. Please visit our Stand No 13 where you will learn about the latest technology from manufacturers such as Biolog, Neogen, Pro-lab and Interscience.

Edwards Group
Website: www.edwardssco.com.au
Edwards (est. since 1961), manufactures and distributes general laboratory equipment and related consumables. To further our commitment to you, our customer, we have recently partnered with Micromedia increasing our current product portfolio of benchtop consumables for all applications to now also offer a comprehensive range of prepared & dehydrated culture media with customisation being a speciality. Phone today and discuss your individual needs 1800 024 407 or micro@edwardssco.com.au

Genetic Signatures
Website: www.geneticsignatures.com
Genetic Signatures develops EasyScreen® multiplex, real-time PCR kits for rapid, sensitive infectious disease detection. Our universal sample preparation technology allows for uniform nucleic acid isolation with reduced contamination risk, and can be coupled with our automated extraction and liquid handling platform for preparation of high-throughput 384-well PCR plate formats.

Helena Laboratories
Website: www.helena.com.au
Helena Laboratories (Australia) Pty. Ltd. has been providing specialized pathology products to Australian laboratories for over 30 years. This year we are excited to announce the launch of an innovative range of Microbiology stainers from Dagatron. The product range includes individual AFB (TB) Stainers, an AFB (TB) Multi Stainer, a Gram Auto Stainer and a GRAM/AFB (TB) Dual Stainer. Please take the opportunity to visit our stand to view and discuss how these new stainers can be incorporated into your laboratory.

Hologic Australia
Website: www.hologic.com/en/
Hologic, Inc. is a leading developer, manufacturer and supplier of premium diagnostic and medical imaging systems and surgical products dedicated to serving the healthcare needs of women. Hologic’s core business units are focused on mammography and breast biopsy, radiation treatment for early-stage breast cancer, cervical cancer screening, and treatment for menorrhagia, osteoporosis assessment, and preterm birth screening.
Illumina Australia
Website: www.illumina.com
Illumina’s next-generation sequencing (NGS) takes you inside microbiology. Put the big picture into focus by revealing the smallest of details.
- Characterise un-culturable organisms
- Develop new strategies to control outbreaks
- Monitor host-pathogen interactions
NGS is opening new doors in microbial genomics.
Through the power and high resolution of Illumina technology, you can now understand the genetic makeup of organisms that were previously impossible to study – helping you examine microbial biological functions, track genetic changes, monitor food sources and more.
We look forward to seeing you at the Illumina booth at ASM.
Don’t miss out on our Sunday afternoon workshop from 1-4pm.

In Vitro Technologies
Website: www.invitro.com.au
In Vitro Technologies, a JJ Richards company, specialises in the distribution, marketing sales and support of quality systems and products for the diagnostics, medical, life science and industrial segments of the Australian and New Zealand market.
The Diagnostics Division represents leading global niche market companies in biochemistry, immunology, haematology, microbiology, molecular diagnostics, veterinary and point of care systems and test kits.
For microbiology, In Vitro Technologies represents 77Elektronika UriSed and LabUMat automated urine analysers for moderate to high workload urine screening, allowing better use of resources.

LAFTech
Website: www.laftech.com.au
LAFTech has been providing Laboratory Contamination control equipment, certification, and Bio-decontamination since 1987. It is the leading supplier and NATA certifier of Contamination control equipment and associated clean room/containment environments within Australia. LAFTech has supplied and installed over 2000 Biological Safety cabinets and annually issues approximately 4000 NATA endorsed test certificates. Based in Australia we are Australian owned and run, with head office located in Melbourne and a satellite office in QLD.

Roche Diagnostics Australia
Website: www.roche-australia.com
At Roche, innovating healthcare is at the core of our business strategy through leading pathology solutions and supporting patient management. Our expanding knowledge and understanding of disease mechanisms leads us to deliver significant benefits to patients and healthcare professionals. Roche’s diagnostic instruments and reagents help doctors detect diseases, select appropriate treatments and monitor patients’ responses to care.

Tecan Australia
Website: www.tecan.com
Tecan is a leading global provider of laboratory instruments and solutions in biopharmaceuticals, forensics and clinical diagnostics.
We specialize in the development, production and distribution of automated workflow solutions for laboratories in the life sciences sector. Clients include pharmaceutical and biotechnology companies, university research departments, forensic and diagnostic laboratories.

Zoetis
Website: www.zoetis.com.au
Zoetis is the leading animal health company, dedicated to supporting its customers and their businesses. Building on more than 60 years of experience in animal health, Zoetis discovers, develops, manufactures and markets veterinary vaccines and medicines, complemented by diagnostic products and genetic tests and supported by a range of services.
SIG AND ASSOCIATED MEETINGS

MONDAY

Cosmetics & Pharmaceuticals SIG AGM
Date: Monday July 12th 2015
Time: 10:00-10:30am
Room: Studio 2 & 3

Parasitology & Tropical Medicine SIG AGM
Date: Monday July 12th 2015
Time: 10:00-10:30am
Room: Eureka Room

Clinical Serology SIG AGM
Date: Monday July 12th 2015
Time: 12:30-13:10
Room: Ballroom 3

Microbial Informatics SIG AGM
Date: Monday July 12th 2015
Time: 12:30-13:10
Room: Ballroom 2

History SIG AGM
Date: Monday July 12th 2015
Time: 13:10-13:50
Room: Ballroom 3

Molecular Microbiology SIG AGM
Date: Monday July 12th 2015
Time: 13:10-13:50
Room: Ballroom 2

WEDNESDAY

Student SIG AGM
Date: Wednesday July 15th 2015
Time: 12:40 – 13:00
Room: Ballroom 3

Mycology SIG AGM
Date: Wednesday July 15th 2015
Time: 12:40 – 13:00
Room: Studio 2&3

CDS User Group Meeting
Date: Wednesday July 15th 2015
Time: 12:40 – 13:00
Room: Ballroom 2

Education SIG AGM
Date: Wednesday July 15th 2015
Time: 12:40 – 13:00
Room: Eureka Room

Annual General Meeting
Date: Tuesday July 13th 2015
Time: 12:30-13:30
Room: Studio 2 & 3
REGISTER AND SUBMIT YOUR ABSTRACT NOW
www.bacpath2015.org

BacPath 13: Molecular Analysis of Bacterial Pathogens
Silverwater Resort, Phillip Island - VIC
27 - 30 September 2015

CONFIRMED INVITED SPEAKERS:
Elizabeth Hartland - The University of Melbourne, Australia
Ian Henderson - University of Birmingham
Jay Hinton - University of Liverpool
Meta Kuehn - Duke University, USA
Denise Monack - Stanford University, USA
PROGRAM

SUNDAY 12TH JULY

WORKSHOP:
CAPSIG: Measurement of Uncertainty in the Microbiological Test Lab
8:00am - 10:00am
Ballroom 2

WORKSHOP:
Update on Antimicrobials Resistance: Gram Positive/Gram Negative & Methods of Testing
8:00am - 10:00am
Ballroom 3

WORKSHOP:
Imaging - Bio-imaging and Single Cell Interrogation
8:00am - 10:00am
Studio 2

WORKSHOP:
Proteomics - A Microbial Proteomics Masterclass
8:00am - 10:00am
Studio 3

WORKSHOP:
Bio-informatics Software Carpentry
8:00am - 12:00pm
Australian National University

Morning Tea
10:00am - 10:30am
Foyer

WORKSHOP:
CAPSIG – Measurement of Uncertainty in the Microbiological Test Lab (continued)
10:30am - 1:00pm
Ballroom 2

WORKSHOP:
Update on Antimicrobials Resistance: Gram Positive/Gram Negative & Methods of Testing (continued)
10:30am - 12:00pm
Ballroom 3

WORKSHOP:
Imaging – Bio-imaging and Single Cell Interrogation (continued)
10:30am - 12:00pm
Studio 2

WORKSHOP:
Proteomics - A Microbial Proteomics Masterclass (continued)
10:30am - 12:00pm
Studio 3

Break
12:00pm - 1:00pm

Illumina Workshop
1:00pm – 4:00pm
Sponsored by illumina

With afternoon tea
SUNDAY 12TH JULY

Public Lecture: Guts to Great Oceans
4:00pm - 5:30pm
The Academy of Science/Shine Dome

Drinks and nibbles will be provided before the lecture

Janet Jansson
Stephen Giovannoni
Mike Manefield

Welcome & Awards Ceremony
6:00pm - 6:30pm
Ballroom 2 & 3

Bazeley Oration
6:30pm - 7:30pm
Ballroom 2 & 3
Chair: Eugene Maraskovsky
Yoshihiro Kawaoka
Influenza or Ebola – which is the greater threat?

Welcome Function
7:30pm - 9:30pm
Exhibition Hall & Foyer
MONDAY 13TH JULY

Nancy Millis Mentoring Student Breakfast
7:15am - 8:30am  Eureka Room

Plenary 1
8:30am - 9:15am  Ballroom 2 & 3
Chair: Jon Iredell
Stefan Schwarz
New antimicrobial resistance genes in MRSA and other staphylococci of animal origin  abs#2

Plenary 2
9:15am - 10:00am  Ballroom 2 & 3
Chair: Julian Rood
Jorge Galan
Salmonella Typhi and typhoid fever: new insights into an old disease  abs#3

Morning Tea
10:00am - 10:40am  Exhibition Hall & Foyer

SIG Meeting: Cosmetics and Pharmaceuticals (CAPSIG)
10:10am - 10:40am  Studio 2 & 3
SIG Meeting: Parasitology and Tropical Medicine
10:10am - 10:40am  Eureka Room

Regulation & Risk in Microbiology
10:40am - 12:20pm  Ballroom 3
Chair: Greg James

10:40  Randy Hutt
How to Handle Excursions in Environmental (EM) and Personnel Monitoring (PM) in an Aseptic Processing Plant  abs#4

11:00  Kathryn Rusbridge
Case studies involving validation/verification difficulties  abs#5

11:20  Tran Tang
Department of Agriculture, Animal and Biological Import Assessment Branch. Biosecurity Risks and Biological Products  abs#6

11:40  Andrew Lattimore
Preparing for Regulatory &/or Customer Compliance Audits  abs#7

12:00  Paul Keese
Back to the Future: Reinventing a New Paradigm for Risk Assessment  abs#8

12:10  Tuflikha Putri
Modelling Microbial Ecology of Geobacillus spp. in Dairy Powder Plants  abs#9
### Structural Microbiology

**10:40am - 12:20pm**  
**Ballroom 2**

Chair: Naresh Verma

10:40 **Marc Kvansakul**  
Structural insight into vaccinia virus mediated inhibition of host cell death  
*abs#10*

11:00 **Sheena McGowan**  
Aminopeptidase inhibitors as new antimalarial agents  
*abs#11*

11:20 **Charles Collyer**  
Type 2 gingipain adhesins act in bacterial invasion  
*abs#12*

11:40 **Michael Landsberg**  
Seeing bacterial ABC toxins at near-atomic resolution by cryo-EM  
*abs#13*

12:00 **Andrew Robinson**  
Single-molecule imaging of DNA polymerase V in *E. coli*: mutagenesis is repressed by membrane binding and is induced by antibiotics  
*abs#14*

12:10 **Elizabeth Harry**  
Coordinating bacterial cell division with nutrient availability: a role for glycolysis  
*abs#15*

### Human Microbial Ecology

**10:40am - 12:20pm**  
**Studio 2 & 3**

Chair: Cynthia Whitchurch

10:40 **Mark Read**  
Simulating the Influence of Diet on Intestinal Microbiome Composition  
*abs#16*

11:00 **Laura Weyrich**  
Untangling the evolutionary history of infectious diseases and the human microbiome using Neandertal dental calculus  
*abs#17*

11:20 **Eric Reynolds**  
Chronic Periodontitis – a destructive inflammatory disease associated with dysbiosis to produce a pathogenic polymicrobial biofilm  
*abs#18*

11:40 **Catherine Burke**  
The microbiome of skin and chronic wounds in type II diabetes  
*abs#19*

12:00 **Edwina Buckle**  
Pyocyanin secreted by *Pseudomonas aeruginosa* inhibits growth of cystic fibrosis isolates of *Staphylococcus aureus* and *Escherichia coli*, but not *Burkholderia cenocepacia*  
*abs#20*

12:10 **Erin Shanahan**  
Confirmation of a Duodenal Mucosa-Associated Microbiota  
*abs#21*

### Lunch

**12:20pm - 1:50pm**  
**Exhibition Hall & Foyer**

**SIG Meeting: Clinical Serology and Molecular**  
**12:30pm - 1:10pm**  
**Ballroom 3**

**SIG Meeting: Microbial Bioinformatics**  
**12:30pm - 1:10pm**  
**Ballroom 2**

**Nancy Millis Student Mentoring Lunch**  
**12:30pm - 1:50pm**  
**Eureka Room**

**SIG Meeting: Food Microbiology**  
**12:45pm - 1:25pm**  
**Studio 3**
## MONDAY 13TH JULY

<table>
<thead>
<tr>
<th>Event</th>
<th>Time</th>
<th>Room</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ECR Mentoring Lunch</strong></td>
<td>12:45pm - 1:50pm</td>
<td>Studio 2</td>
</tr>
<tr>
<td><strong>SIG Meeting: History</strong></td>
<td>1:10pm - 1:50pm</td>
<td>Ballroom 3</td>
</tr>
<tr>
<td><strong>SIG Meeting: Molecular Microbiology</strong></td>
<td>1:10pm - 1:50pm</td>
<td>Ballroom 2</td>
</tr>
<tr>
<td><strong>Infection Control &amp; Vaccination</strong></td>
<td>1:50pm - 3:30pm</td>
<td>Ballroom 3</td>
</tr>
<tr>
<td>Chair: Tim Newsome</td>
<td></td>
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</tr>
<tr>
<td>1:50 Guna Karupiah</td>
<td>1:50pm</td>
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<tr>
<td>Mechanisms through which live viral vaccines generate superior long-lived humoral immunity</td>
<td>abs#22</td>
<td></td>
</tr>
<tr>
<td>2:10 Karen Laurie</td>
<td>2:10pm</td>
<td></td>
</tr>
<tr>
<td>The time interval between infections and viral hierarchies are determinants of viral interference following influenza virus infection in a ferret model</td>
<td>abs#23</td>
<td></td>
</tr>
<tr>
<td>2:30 Carl Kirkwood</td>
<td>2:30pm</td>
<td></td>
</tr>
<tr>
<td>Pattern of rotavirus strains circulating in Australia post vaccine introduction</td>
<td>abs#24</td>
<td></td>
</tr>
<tr>
<td>2:50 Richard Strugnell</td>
<td>2:50pm</td>
<td></td>
</tr>
<tr>
<td>Initiating and sustaining immune responses – lessons from infection</td>
<td>abs#25</td>
<td></td>
</tr>
<tr>
<td>3:10 Cynthia Whitchurch</td>
<td>3:10pm</td>
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<tr>
<td>Stigmatic social behaviours facilitate the active expansion of Pseudomonas aeruginosa interstitial biofilms</td>
<td>abs#26</td>
<td></td>
</tr>
<tr>
<td>3:20 Anjali Gowripalan</td>
<td>3:20pm</td>
<td></td>
</tr>
<tr>
<td>Honing the oncolytic weapon: Increased invasiveness of vaccinia virus-infected cells</td>
<td>abs#27</td>
<td></td>
</tr>
</tbody>
</table>

| **Industrial Microbiology**                                          | 1:50pm - 3:30pm    | Ballroom 2 |
| Chair: Colin Scott                                                  |                    |            |
| 1:50 Eddy Smid                                                      | 1:50pm             |            |
| Linking microbial community structure to functionality of a complex mesophilic dairy starter | abs#28             |
| 2:10 Ashley Franks                                                 | 2:10pm             |            |
| Getting dirty - application of soil microbiology to shock and heal  | abs#29             |
| 2:30 Lars Nielsen                                                  | 2:30pm             |            |
| Opening Pandora’s Box                                              | abs#30             |
| 2:50 Sally Gras                                                     | 2:50pm             |            |
| The selective control of microbial growth in complex communities: from functional foods and colonic bacteria to bacteriophage and wastewater foaming | abs#31             |
| 3:10 Sabrina Beckmann                                              | 3:10pm             |            |
| Hot-wiring microbial communities for enhanced biogas production with an organic semiconductor | abs#32             |
| 3:20 Van Ho                                                        | 3:20pm             |            |
| A fast screening method for selecting Cheddar cheese flavour adjuncts based on detection of odour-active compounds | abs#33             |
# MONDAY 13TH JULY

## New and Unusual Microbes

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
<th>Title</th>
<th>Abs#</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:50</td>
<td>Chantal Abergel</td>
<td>Mimivirus and Mimiviridae revive the historical debate on the true nature of viruses</td>
<td>34</td>
</tr>
<tr>
<td>2:10</td>
<td>Jan Slapeta</td>
<td>The poorly known sister groups of the phylum Apicomplexa</td>
<td>35</td>
</tr>
<tr>
<td>2:30</td>
<td>John Fuerst</td>
<td>Planctomycetes - new models for microbial cells and their activities</td>
<td>36</td>
</tr>
<tr>
<td>2:50</td>
<td>Mike Dyall-Smith</td>
<td>Not available at time of print</td>
<td>37</td>
</tr>
<tr>
<td>3:10</td>
<td>Damien Stark</td>
<td>Molecular epidemiology of imported cases of leishmaniasis in Australia from 2008-2014</td>
<td>38</td>
</tr>
<tr>
<td>3:20</td>
<td>Ratiyakorn Singwisut</td>
<td>Isolation of thermophilic cellulolytic bacteria from sugarcane bagasse pile</td>
<td>39</td>
</tr>
</tbody>
</table>

### Afternoon Tea

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Location</th>
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</thead>
<tbody>
<tr>
<td>3:30</td>
<td>Afternoon Tea</td>
<td>Exhibition Hall &amp; Foyer</td>
</tr>
</tbody>
</table>

## Diagnostic Microbiology

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
<th>Title</th>
<th>Abs#</th>
</tr>
</thead>
<tbody>
<tr>
<td>4:00</td>
<td>Linda Hueston</td>
<td>Emerging and re-emerging viruses and their threat to human health</td>
<td>40</td>
</tr>
<tr>
<td>4:20</td>
<td>Tom Olma</td>
<td>Automation of Agar Based Diagnostic Bacteriology - morphing into One Microbiology</td>
<td>41</td>
</tr>
<tr>
<td>4:40</td>
<td>Chong Ong</td>
<td>Vancomycin-Dependent Enterococci – An Overview</td>
<td>42</td>
</tr>
<tr>
<td>5:00</td>
<td>Anindita Das</td>
<td>Improving microbiological diagnosis of prosthetic orthopaedic device infections: role of sonication</td>
<td>43</td>
</tr>
<tr>
<td>5:20</td>
<td>Peter Njuguna</td>
<td>Novel qPCR assays for the detection of causative agents of viral and bacterial meningitis</td>
<td>44</td>
</tr>
<tr>
<td>5:30</td>
<td>Joanne Letchford</td>
<td>Diagnostic Microbiology in Cambodia</td>
<td>45</td>
</tr>
</tbody>
</table>
MONDAY 13TH JULY

Synthetic Biology & Biotechnology
4:00pm - 5:40pm
Chair: Lars Nielsen

4:00 Sakkie Pretorious
Role of Yeast 2.0 in the advancement of Synthetic Biology

4:20 Claudia Vickers
From basic biology to industrial biotechnology: Lessons learned through engineering synthetic biological networks and pathways

4:40 Colin Scott
Evolution of new enzyme function in bacteria: lessons from herbicide catabolism

5:00 Monica Gerth
Discovery and design of microbial chemoreceptors: New parts for synthetic biology

5:20 Ian Macreadie
Use of yeast to screen for chemopreventatives for Alzheimer’s Disease

5:30 Lucie Semene
Synthetic Biology Mining: Examining Community Dynamics of Mixed Species Electrogenic Biofilms

Marine Microbiology
4:00pm - 5:40pm
Chair: Cath Burke

4:00 Stephen Giovannoni
Systems Biology and Ecology of Streamlined Bacterioplankton

4:20 Madeleine van Oppen
Coral-associated viruses: pathogens, mutualists, and agents of evolution?

4:40 Federico Lauro
The Rise of Citizen Oceanography: Metagenomic Advances in the Interpretation of the Marine Microbiome

5:00 Sophie Mazard
Defining the differences between symbiotic and free-living cyanobacteria

5:20 Lauren Messer
Biogeography of nitrogen fixers in the Australian coastal ocean

5:30 Zoe Dyson
Evaluation of potential phage based methods to control foam in wastewater treatment systems

Break
5:40pm - 5:50pm

Frank Fenner Lecture
5:50pm – 6:15pm
Chair: Mike Manefield
Gene Tyson
Expanding our view of the microbial metabolic and phylogenetic diversity through metagenomics

Lyn Gilbert Lecture
6:15pm - 6:40pm
Chair: Jon Iredell
Theo Sloots
From Diagnostics to Research in Microbiology
MONDAY 13TH JULY

Turkish Society for Microbiology
6:40pm - 7:00pm
Ballroom 2 & 3

Poster Session A
7:00pm - 8:30pm
Exhibition Hall & Foyer

Student & ECR Night
9:00pm - late
Bent Spoke Brewery

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E: winterhalter.adam@sysmex.com.au  M: 0431-176-005

We Believe the Possibilities
TUESDAY 14TH JULY

Plenary 3
8:30am - 9:15am
Chair: Tim Newsome

**Chantal Abergel**
The exciting universe of giant viruses

Plenary 4
9:15am - 10:00am
Chair: Nick Coleman

**Stephen Giovannoni**
Predicting Long-Term Impacts of Ocean Desertification on Microbial Plankton Communities

Morning Tea
10:00am - 10:30am

Epidemiology
10:30am - 12:10pm
Chair: Andrew Gordon

10:30 **Stefan Schwarz**
Molecular epidemiology of ESBL genes in *Escherichia coli*: dissemination of resistant clones and resistance plasmids

10:50 **Grant Hill-Cawthorne**
Using genomics to inform epidemiology

11:10 **Sebastian Van Hal**
Enterococcus faecium epidemiology: the changing paradigm

11:30 **Rosemarie Sadsad**
The role of whole genome sequencing in hospital outbreak surveillance and investigation

11:50 **Ruiting Lan**
A genomic portrait of evolution and epidemic spread of a recently emerged multidrug resistant *Shigella flexneri* clone in China

12:00 **David Cleary**
From Genomes to Communities: analyses of the polymicrobial composition in the upper respiratory tract
TUESDAY 14TH JULY

Host Pathogen Interactions I
10:30am - 12:10pm
Chair: Michael Frese

10:30 Barry Slobedman
Can We Predict Human Cytomegalovirus Reactivation In Naturally Infected Haematopoietic Stem Cell Transplant Recipients?
10:50 Markus Hofer
A tale of two signals: the divergent roles of type I interferon signalling factors in the antiviral immune response.
11:10 Maria Liaskos
The role of Helicobacter pylori outer membrane vesicles in inflammation and immunity
11:30 Jamie Triccas
Improving tuberculosis control by targeting the pathogen life cycle
11:50 Jaelle Brealey
Respiratory syncytial virus and bacterial co-infection of the paediatric respiratory tract
12:00 Noorul Bishara Marzook
The role of beta- and gamma-actin isoforms in vaccinia virus actin tail morphology

Molecular Genetics and Evolution
10:30am - 12:10pm
Chair: Dena Lyras

10:30 Miles Davenport
Mutation, recombination, and selection in HIV infection.
10:50 Edward Holmes
The Evolution of Viral Emergence
11:10 Giel Van Dooren
Regulated transport of the essential amino acid arginine into Toxoplasma parasites
11:30 Slade Jensen
Methicillin-resistant Staphylococcus aureus within Host Evolution
11:50 Sanja Trifkovic
The Dynamics of Influenza A virus Reassortment
12:00 Hannah Read
Through a Mouse, Brightly: in vivo experimental evolution of the pathogenic bacterium Citrobacter rodentium

Lunch
12:10pm - 1:30pm

Annual General Meeting
12:30pm - 1:30pm
TUESDAY 14TH JULY

BD Awards Symposium
1:30pm - 3:00pm
Chair: Cheryl Power

1:30  Lucy Furfaro
In vitro activity of solithromycin and metabolites, CEM-214 and N-Acetyl-CEM-101, against 100 clinical Ureaplasma spp. isolates.  abs#80

1:45  Amelia Hynen
Explosive Cell Lysis in Pseudomonas aeruginosa Biofilms  abs#81

2:00  Hayden Thain
Understanding regulation of qacR within Staphylococcus aureus will allow for sidestepping of antimicrobial resistance  abs#82

2:15  Sushama Telwatte
Silent Mutations in HIV-1 Reverse Transcriptase in Subtype B HIV-1 containing D67N and K70R Drug Resistance Mutations Restore Viral Fitness and AlleviateIndel Formation  abs#83

2:30  Chris Atkinson
Characterization of Macrolide Resistance in Nontypeable Haemophilus influenzae  abs#84

2:45  Agathe Colmant
Discovery of a new species of mosquito specific flavivirus in Australia using an innovative sequence-independent virus detection system  abs#85

Afternoon Tea
3:00pm - 3:30pm
Emerging Pathogens
3:30pm - 5:10pm
Chair: Grant Hill-Cawthorne

3:30  Greg Moseley
Targeting the pathogenic interaction of lyssaviruses  abs#86

3:50  Jason Roberts
Enteroviruses Associated with Acute Flaccid Paralysis in Oceania - Results from a Decade of Surveillance  abs#87

4:10  Cameron Webb
Future mosquito-borne disease threats in Australia  abs#88

4:30  Greg James
Laboratory investigation of emerging infections and Ebola virus  abs#89

4:50  Michael Nissen
Current status of Ebolavirus vaccine development  abs#90
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Speaker</th>
<th>Title</th>
<th>Abs#</th>
</tr>
</thead>
<tbody>
<tr>
<td>3:30</td>
<td>Molecular Pathogenesis</td>
<td>Jorge Galan</td>
<td>Salmonella Typhimurium and intestinal inflammation: a pathogen centric view</td>
<td>#91</td>
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<tr>
<td>3:50</td>
<td></td>
<td>Victoria Korolik</td>
<td>Bacterial chemotaxis and changing paradigms</td>
<td>#92</td>
</tr>
<tr>
<td>4:10</td>
<td></td>
<td>Charlene Kahler</td>
<td>Acquisition of the capsule locus in Neisseria meningitidis and the loss of N acetylgalactosamine biosynthesis</td>
<td>#93</td>
</tr>
<tr>
<td>3:30</td>
<td></td>
<td>Peter Waterhouse</td>
<td>Vigour and Virus Hypersusceptibility: the all-natural, gym-junkie, nude mouse, plant</td>
<td>#94</td>
</tr>
<tr>
<td>4:50</td>
<td></td>
<td>Eleanor Latomanski</td>
<td>The Coxiella burnetii effector protein Cig57 subverts clathrin-mediated endocytosis within the human host cell</td>
<td>#95</td>
</tr>
<tr>
<td>5:00</td>
<td></td>
<td>Jal Tree</td>
<td>Understanding the roles of non-coding RNAs in Enterohaemorrhagic E. coli pathogenesis</td>
<td>#96</td>
</tr>
<tr>
<td>3:30</td>
<td>Animal Diseases</td>
<td>Peter Walker</td>
<td>Connecting North and South: Landscape genomics of arbovirus infections of livestock in Australia</td>
<td>#97</td>
</tr>
<tr>
<td>3:50</td>
<td></td>
<td>Michael Ward</td>
<td>Bovine O157 supershedders: fact or fiction?</td>
<td>#98</td>
</tr>
<tr>
<td>4:10</td>
<td></td>
<td>Lee Skerratt</td>
<td>One health approaches to animal conservation</td>
<td>#99</td>
</tr>
<tr>
<td>4:30</td>
<td></td>
<td>Paul Hick</td>
<td>Epidemiological approaches to investigation of viral diseases of aquatic animals</td>
<td>#100</td>
</tr>
<tr>
<td>4:50</td>
<td></td>
<td>Carla Giles</td>
<td>Characterisation and comparative analyses of the Equine adenovirus 1 and 2 complete genome sequences</td>
<td>#101</td>
</tr>
<tr>
<td>5:00</td>
<td></td>
<td>Lechelle van Breda</td>
<td>Antimicrobial resistance of Escherichia coli isolated from piglets in South Eastern Australian piggeries.</td>
<td>#102</td>
</tr>
</tbody>
</table>

**Poster Session B**

5:10pm - 6:30pm  
Exhibition Hall & Foyer

**Rubbo Oration**

6:30pm - 7:30pm  
Ballroom 2 & 3  
Chair: Dena Lyras  
Janet Jansson  
Multi-omics of the human gut microbiome  
Abs#103

**Rubbo Dinner**

7:30pm - 10:00pm  
Eureka Room

**After Party**

8:00pm - late  
Lucky’s SpeakEasy Bar
WEDNESDAY 15TH JULY

Plenary 5
9:00am - 9:45am
Chair: Dee Carter

**Judith Berman**
Genome dynamics and drug resistance
abs#104

Snowdon Oration
9:45am - 10:30am
Chair: Jack Wang

**Chris Baggoley**
Australian and international response to public health threats
abs#105

Sponsored by

Morning Tea
10:30am - 11:00am
Exhibition Hall & Foyer

Antimicrobials and Resistance
11:00am - 12:40pm
Chair: Ruth Hall

11:00 **Rowena Martin**
Mechanisms underlying patterns of inverse drug resistance in the human malaria parasite *Plasmodium falciparum*
abs#106

11:20 **Melissa Brown**
Bacterial antimicrobial resistance – how compounds are extruded from cells by secondary multidrug transporters
abs#107

11:40 **Dale Dominey-Howes**
Antimicrobial resistance as a disaster risk problem - insights from an outsiders perspective
abs#108

12:00 **Mark Blaskovich**
Why are new antibiotics so hard to find?
abs#109

12:20 **Emma Peel**
Cathelicidins in the Tasmanian devil (*Sarcophilus harrisii*)
abs#110

12:30 **Christine Roder**
Novel antimicrobial therapy that targets selenium metabolism to treat *Clostridium difficile* infection
abs#111
Ecosystem Health
11:00am - 12:40pm
Chair: Nick Coleman

11:00  **Athol Klieve**
Kangaroos and cattle; anaerobic fermentation with and without methane production

11:20  **Gunjan Pandey**
Degradation of insensitive munition ingredient 2,4-dinitroanisole by bacteria

11:40  **Ian Anderson**
Impacts of climate change factors on eucalypt ectomycorrhizal fungi

12:00  **Martina Doblin**
Regimes of temperature variability for microbial adaptation to ocean change

12:20  **Thomas Jeffries**
Unravelling microbial bioremediation of organophosphates: a metagenomic systems-biology approach

12:30  **Jen Wiltshire**
Microscopic interactions with macroscopic effects: Do microorganisms create plant diversity in rainforests?

Host-Pathogen Interactions II
11:00am - 12:40pm
Chair: Alex Idnurm

11:00  **Peter Solomon**
Understanding the Parastagonospora nodorum – wheat interaction; is it as simple as we think?

11:20  **Thomas Naderer**
Macrophage-pathogen interactions: a metabolic perspective

11:40  **Rohit Jain**
Intravital imaging of leukocyte homing during Cerebral Malaria

12:00  **Alex Andrianopoulos**
Mechanisms for surviving within the host - an emerging fungal pathogen’s perspective

12:20  **Sophia Lev**
Fungal inositol pyrophosphate IP7 is crucial for host-pathogen interaction and virulence

12:30  **Tracey Steinrucken**
Endophytic pathogens, water stress and dieback in an invasive tree

SIG Meeting: Students (STUDSIG)
12:45pm - 1:20pm

CDS User Group Meeting
12:45pm - 1:20pm

SIG Meeting: Mycology
12:45pm - 1:20pm

SIG Meeting: EDUSIG
12:45pm - 1:20pm
### WEDNESDAY 15TH JULY

**Poster Session C**  
1:20pm - 2:00pm  
Exhibition Hall & Foyer

#### Proffered Papers I

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<tr>
<th>Time</th>
<th>Speaker</th>
<th>Title</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>2:00</td>
<td>Shakeel Mowlaboccus</td>
<td>Molecular Epidemiology and Antigen Profiling of <em>Neisseria meningitidis</em> in Western Australia from 2000 to 2014</td>
<td>Ballroom 3</td>
</tr>
<tr>
<td>2:10</td>
<td>Meghan Jones</td>
<td>Microevolution of <em>E. coli</em> O157:H7 in a herd of Australian cattle</td>
<td></td>
</tr>
<tr>
<td>2:20</td>
<td>Ayalew Mekonnen</td>
<td>Development and validation of a serotype specific quantitative PCR assay for detection of equine adenovirus-2</td>
<td></td>
</tr>
<tr>
<td>2:30</td>
<td>Jessica Knight</td>
<td>Biofilms – hidden environmental reservoir of antibiotic resistant microorganisms in ICU</td>
<td></td>
</tr>
<tr>
<td>2:40</td>
<td>Keith Stanley</td>
<td>Sequencing of PCR positive <em>Giardia</em> and <em>Campylobacter</em> reveals novel genotypes</td>
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#### Proffered Papers II

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<th>Speaker</th>
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<tbody>
<tr>
<td>2:00</td>
<td>David Tscharke</td>
<td>Leaky lytic gene expression is an integral part of herpes simplex virus latency</td>
<td>Ballroom 2</td>
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<tr>
<td>2:10</td>
<td>Nicholas Coleman</td>
<td>Engineering <em>E.coli</em> for growth on organochlorine pollutants</td>
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<tr>
<td>2:20</td>
<td>Benjamin Raymond</td>
<td>A systems approach for the identification of proteins from <em>Mycoplasma hyopneumoniae</em> required for biofilm formation</td>
<td></td>
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<tr>
<td>2:30</td>
<td>Ed Fox</td>
<td>Ecology of <em>Listeria</em> species on Australian farm environments suggests a key role for aquatic niches in contamination routes</td>
<td></td>
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<tr>
<td>2:40</td>
<td>Edwina Buckle</td>
<td>Pyocyanin secreted by <em>Pseudomonas aeruginosa</em> inhibits growth of cystic fibrosis isolates of <em>Staphylococcus aureus</em> and <em>Escherichia coli</em>, but not <em>Burkholderia cenocepacia</em></td>
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# AMS Proffered Papers

**2:00** Desmarini Desmarini  
Understanding phosphate acquisition strategies in *Cryptococcus neoformans*  
abs#134  

**2:10** Barbara Drigo  
What will climate change mean for infectious disease? The soil perspective  
abs#135  

**2:20** Cecilia Li  
Role of the inositol polyphosphate kinase Ipk1 in the pathogenicity of *Cryptococcus neoformans*  
abs#136  

**2:30** Jeff Powell  
Drivers’ Disconnect: Deterministic processes vary during community assembly for ecologically dissimilar taxa  
abs#137  

**2:40** Linda Henderson  
Copper (II) Lead (II) and Zinc (II) inhibit the growth, reproduction and rate of attachment to organic substrates of four zoosporic fungi species from soils of NSW  
abs#138  

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### Afternoon Tea

2:50pm - 3:20pm  
Exhibition Hall & Foyer

# Public Health Microbiology

**3:20** Amy Jennison  
Challenges and opportunities: whole genome sequencing in public health microbiology  
abs#139  

**3:40** Peter Collignon  
One Health and Superbugs; the ever growing threat from foods and water.  
abs#140  

**4:00** Matthew Watts  
*Annacalia algerae* microsporidial myositis in Australia  
abs#141  

**4:20** Namraj Goire  
Molecular strategies for gonococcal antimicrobial resistance surveillance in Australia  
abs#142  

**4:40** Louise Causer  
Operational performance of a new molecular-based point-of-care test for diagnosis of Chlamydia trachomatis and Neisseria gonorrhoeae infection: concordance with conventional laboratory testing  
abs#143  

**4:50** Vivek Pande  
*Salmonella* Typhimurium shedding and egg contamination in experimentally infected laying hens  
abs#144
WEDNESDAY 15TH JULY

Mobile DNA
3:20pm - 5:00pm
Chair: Slade Jensen

3:20 **Sally Partridge**
Identifying the true ftrS end of ISCR1?  
abs#145

3:40 **Stephen Kwong**
Replication of staphylococcal multiresistance plasmids  
abs#146

4:00 **Julian Rood**
Functional analysis of the conjugation system of *Clostridium perfringens*  
abs#147

4:20 **Michael Gillings**
Exposure to sub-clinical concentrations of antibiotics induces genomic and phenotypic changes and promotes antibiotic resistance  
abs#148

4:40 **Sam Manna**
Horizontal gene transfer of Chlamydial tRNA modification enzymes to parasitic protozoa: An untapped resource of putative drug targets?  
abs#149

4:50 **Chun Hoong Ho**
Stressors and Prophage Stability in *Lactococcus lactis*  
abs#150

Omics and Systems Biology
3:20pm - 5:00pm
Chair: Stuart Cordwell

3:20 **Judith Berman**
The dynamics of chromosome components that promote genome stability: Centromeres, Telomeres and Origins of replication  
abs#151

3:40 **Megan McDonald**
High gene and genomic variation revealed in the re-sequencing of 13 isolates of the fungal wheat pathogen *Zymoseptoria tritici*  
abs#152

4:00 **Marc Wilkins**
Discovering the Protein Interaction Code: post-translational modifications that modulate protein-protein interactions  
abs#153

4:20 **James Fraser**
Teaching an old target new trick: Focusing on purine biosynthesis in antifungal development  
abs#154

4:40 **Kylie Brice**
The Koala (*Phascolarctos cinereus*) Faecal Bacterial Microbiome; Does Change in Diet Impact Community Structure?  
abs#155

4:50 **Jacqueline Melvold**
The development of a proteogenomic pipeline to characterize the type VI secretory system (T6SS) of Gram-negative bacteria  
abs#156

Closing Address
5:00pm - 5:15pm

Ballroom 2 & 3
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<td>Novatec NovaLisa™ verus DiaSorin Liaison® XL Assays for the serological detection and diagnosis of <em>Borrelia burgdorferi</em></td>
<td>Bruce Wong</td>
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<td>Allele-specific multiplex–microsphere qPCR genotyping of <em>Campylobacter jejuni</em></td>
<td>Fang Liang</td>
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<td>Same Day MALDI-TOF Identification of Bacteria from Positive Blood Culture Bottles: Evaluation of two Methods</td>
<td>Kerry Varettas</td>
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<td>Evaluation of BioMerieux VITEK MS MALDI-TOF and Bruker biotyper for identification of microorganisms isolated from clinical specimens</td>
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<td>Rapid and accurate identification of pathogens in positive blood cultures by use of Vitek MS Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry System (MALDI-TOF) after short incubation on a solid medium</td>
<td>Belinda Lin</td>
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<td>A comparison of the efficiency of commercially available automated nucleic acid extraction platforms to detect a wide range of respiratory viruses from a bank of clinical samples</td>
<td>Dylan Warby</td>
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<td>Comparison study of The Alere BinaxNOW Legionella Urinary Antigen Card with Quidel Sofia Legionella FIA and Trinity Biotech Uni-Gold Legionella Urinary Antigen PLUS for use in a Microbiology Laboratory setting.</td>
<td>Tina Cai</td>
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<td>Mycoplasma pneumoniae: A Three Year Quality Assurance Review</td>
<td>Raellene Dare-Smith</td>
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<td>The microbial community of Eutherian origin</td>
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<td>High-throughput sequencing of chronic wounds: healing vs non-healing wounds</td>
<td>Irani Rathnayake</td>
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<td>Ian Macreadie</td>
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<td>Cyclic-di-AMP levels in different <em>Lactococcus</em> strains is affected by CdaA and GdpP</td>
<td>Nguyen Nhip</td>
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<td><em>Butyrivibrio</em> and <em>Pseudobutyrivibrio</em> pan-genome analyses provide insight into hemicellulose degradation in the rumen.</td>
<td>Nikola Palevich</td>
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<td>The application of electromicrobiology to reduce sulfur-driven corrosion of the sewer infrastructure</td>
<td>Elizabeth Mathews</td>
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<td>Comparison of microbial communities on pre-painted steel panels from Australia and Malaysia</td>
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<th>Predicted coverage and immuno-safety of a recombinant C-Repeat Region based <em>Streptococcus pyogenes</em> vaccine candidate</th>
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<td><strong>Chris Lidstone</strong></td>
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<td><strong>Alyce Taylor-Brown</strong></td>
<td>Culture-independent genome sequencing and analysis of the chlamydial agent of epitheliocystis in Yellowtail Kingfish</td>
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<td><strong>Kelly Hamonts</strong></td>
<td>A novel polyphasic framework to resolve Yellow Canopy Syndrome paradox</td>
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<th><strong>Sophie Octavia</strong></th>
<th>Delineating community outbreaks of <em>Salmonella enterica</em> serovar Typhimurium using whole genome sequencing</th>
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<td><strong>Honghua Hu</strong></td>
<td>Patient shoe covers: transferring bacteria from the floor onto surgical bed sheets</td>
<td>abs# 226</td>
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<td><strong>Marwan Msarah</strong></td>
<td>Identification of <em>Bacillus cereus</em> emetic toxin gene from Meat Curry</td>
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<td><strong>David McMillan</strong></td>
<td>A culture-independent comparison of bacterial communities on peripheral intravenous catheters and matching skin swabs</td>
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<td><strong>Miranda Sherley</strong></td>
<td>Should we screen women for rectal chlamydia?</td>
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<td><strong>Victoria Wansink</strong></td>
<td>Trends in the microbiological status of ready-to-eat foods in the Australian Capital Territory from 2002-2014</td>
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<td><strong>Belinda Vangchhia</strong></td>
<td><em>Escherichia coli</em> in poultry meat: prevalence, abundance and phylogenetic profiles</td>
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<td><strong>Samantha Burn</strong></td>
<td>Presence of <em>Escherichia coli</em> environmental clades within B1 phylogroup isolated from water catchments in Eastern Australia</td>
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<td><strong>Lara Bereza-Malcolm</strong></td>
<td>Synthetic biology derived biosensors for heavy metal detection</td>
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<td><strong>Anna Gryshyna</strong></td>
<td>Expression of human Fucosyltransferase 3 (FUT3) in <em>Trichoderma reesei</em></td>
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<td><strong>Melanie Duncan</strong></td>
<td>Evolving Resistance to Imatinib: From Molecules to Mice</td>
<td>abs# 236</td>
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**Kate McMillan**  
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**Charlotte Oskam**  
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**Songzhe Fu**  
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**Douglas Chan**  
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**Chris Allen**  
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**Jacqueline Heath**  
P. gingivalis surface-associated molecules interact with the human host  
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**Sarah Croft**  
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**Fiona Sansom**  
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abs# 310

**Tahnee Bridson**  
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abs# 311

**Suan-Sin Foo**  
Pentraxin 3: A key player in shaping arthritogenic alphaviral disease pathogenesis  
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**Matt Johansen**  
The role of intracellular cholesterol within macrophages exposed to Mycobacterium avium subsp. paratuberculosis  
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**Megan Lloyd**  
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**Joshua Newson**  
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**Mohammad Katouli**  
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**Angelin Samuel**  
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**Wen Jun Liu**  
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**Jen-Ren Wang**  
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<td>Brogan Amos</td>
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<td>Biocontrol and plant growth promoting potential of Streptomyces hydrogenans strain DH16</td>
<td>Talwinder Kaur</td>
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<td>Genome editing by CRISPR-Cas9 in pathogenic fungi</td>
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<td>Enhancement of Fatty Acids Production from Saccharomyces cerevisiae by Overexpression of Malic enzyme and Heterologous Expression of Thioesterase from Corynebacterium glutamicum</td>
<td>Seung Kyou You</td>
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<td>Cytokine induction by Cryptococcus strains of varying pathogenicity</td>
<td>Kenya Fernandes</td>
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<td>Dissecting the response to fluconazole in susceptible and resistance strains of Cryptococcus gattii</td>
<td>Aiden Kane</td>
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<td>In-vitro antibacterial activity of underutilized plant crude extracts against food-borne pathogens</td>
<td>Nimsha Weerakkody</td>
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<td>Antibacterial and antibiofilm activity of Alpinia galanga (Galangal) against gram positive bacteria and evaluation of pharmacological safety.</td>
<td>Hansani Karunarathne</td>
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<td>Screening of a bacteriocin-producing Bifidobacterium with antibacterial activity against Clostridium difficile</td>
<td>Methinee Pipatthana</td>
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<td>Potential use of Pediococcus sp. isolated from Thai fermented sausage as probiotics</td>
<td>Panya Nonthasila</td>
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<td>Heterologous expression of an ABC transporter from Clostridium difficile</td>
<td>Chawalit Ngersombat</td>
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<td>Antimicrobial and chlorhexidine mouthwash resistance of dental plaque bacteria</td>
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<td>A preliminary examination of the anti-microbial properties of methanolic fractions obtained from three species of Russula</td>
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<td>Antimicrobial effect of hypertonic saline on Pseudomonas aeruginosa isolates from cystic fibrosis lung infections</td>
<td>Honghua Hu</td>
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<td>Detection of Carbapenemase-producing Enterobacteriaceae in KK Women's and Children's Hospital, Singapore.</td>
<td>Zhenghao Jiang</td>
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<td>Sub-inhibitory doses of aminoglycoside antibiotics induce changes in the phenotype of Mycobacterium abscessus</td>
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<td>Deciphering the molecular mechanisms of ertapenem resistance in Klebsiella pneumoniae</td>
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Influenza or Ebola—which is the greater threat?

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Over the last 100 years, we have experienced four influenza pandemics: one in 1918, one in 1957, one in 1968, and one in 2009. Of these pandemics, the Spanish flu in 1918 was the most devastating, killing more than 40 million people worldwide. In addition to these pandemics, yearly influenza epidemics occur, causing increased morbidity and mortality, particularly in vulnerable populations like the very young and the elderly. Consequently, influenza has an enormous impact on the global economy; huge losses occur as a result of lost work and increased health care expenditures.

By contrast, Ebola virus has only been recognized since 1976, and, until recently, outbreaks caused by this virus had never caused a sizeable number of deaths. However, the recent outbreak in West Africa took hold over a larger, more densely populated urban area and changed our understanding of an outbreak by this virus. I will discuss our recent findings that could lead to the development of antiviral measures to minimize the impact of these viruses.

New antimicrobial resistance genes in MRSA and other staphylococci of animal origin

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With the identification of livestock-associated methicillin-resistant Staphylococcus aureus [LA-MRSA] in 2004, research on that topic has been intensified worldwide during the last decade. In Germany, the research consortium MedVet-Staph, which includes researchers from human and veterinary medicine, has started in 2011. One of the tasks within MedVet-Staph is the identification of novel and uncommon resistance genes.

In the years before MedVet-Staph, several novel and uncommon resistance genes have been identified in LA-MRSA. These comprised the multiresistance gene cfr, which confers combined resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins and streptogramin A antibiotics, the trimethoprim resistance gene dfrK, the lincosamide-pleuromutilin-streptogramin A resistance gene vga(C) and the macrolide-lincosamide-streptogramin B resistance gene erm(T). In 2011, the first and so far only staphylococcal apramycin resistance gene, apmA, has been identified on large multiresistance plasmids. Moreover, small plasmids of an unusual structure carrying the genes apmA or dfrK were identified in 2012. In addition, the pleuromutilin-lincosamide-streptogramin A resistance gene vga(E), first described in 2011, was detected in LA-MRSA from cattle, poultry and food of poultry origin in 2012. A variant of vga(E) was identified on plasmids in porcine coagulase-negative staphylococci. In 2013, the pleuromutilin-lincosamide-streptogramin A resistance gene laz(E) and the spectinomycin resistance gene spd were described. Both genes were part of different types of multiresistance gene clusters of enterococcal origin. These multiresistance gene clusters were initially found in human MRSA ST398 and in MSSA and MRSA ST9 of human and animal origin. Most recently, relics of these multiresistance gene clusters have also been detected in the chromosomal DNA of various coagulase-negative staphylococci. The latest novel resistance gene, the spectinomycin resistance gene spd, has been described in 2014. This gene was detected in MRSA CC398 of various origins from Belgium, The Netherlands, Germany and Austria, but also in porcine MSSA ST433 from Germany. A new variant of the spd gene has recently been identified on structurally diverse plasmids in porcine Staphylococcus hyicus and coagulase-negative staphylococci from Germany.

Salmonella Typhi and typhoid fever: new insights into an old disease

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Salmonella Typhi continues to be a serious global health concern, resulting in more than 200,000 annual deaths. A distinguishing feature of S. Typhi is that it only infects humans, causing a life-threatening systemic infection known as “typhoid fever”. This is in sharp contrast to most other Salmonellae, which can infect a variety of hosts and are usually associated with self-limiting gastroenteritis [i. e., “food poisoning”]. The molecular bases for Salmonella Typhi’s unique pathogenesis and host specificity will be discussed.

How to Handle Excursions in Environmental (EM) and Personnel Monitoring (PM) in an Aseptic Processing Plant

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Control of the environment and personnel activities is critical in an aseptic processing plant to provide sterility assurance. Plants are dependent on Engineering Controls, such as HEPA filtration, frequent air changes per hour, laminar air flow, and use of disinfectants to keep counts of non-viable and viable particulates low. Testing of the environment particulates (viable and non-viable), as well as ensuring personnel monitoring samples have minimal counts, is a vital part of demonstrating control of this environment. This is necessary because Sterility Testing is a destructive finished product test, where only 20-40 samples are tested. A high level of contamination would have to be present to find growth. Low level contamination is not likely to be detected.

The key issue is how to respond to counts found from environmental monitoring (EM) and personnel monitoring (PM), particularly viable ones. The USP, in earlier versions, indicated that investigations were needed for low counts of even 1 CFU, but in May 2014, Version 37 described that individual counts of 1-10 may not be significant. Actually a
count of 15 is deemed to be of concern, requiring investigation. Also, the search for monthly trends was and is recommended for EM and PM, particularly in Grades A and B areas with a 1 to 3% recovery rate, respectively, and to evaluating risk to product quality in those cases. Use of the Fishbone methodology is helpful in reviewing possible root causes. With low numbers of counts, it is sometimes impossible to determine a most probable root cause, and to develop Corrective and Preventive actions (CAPAs). Therefore, we are frequently left with an assessment of whether the risk to product quality is low, medium or high, and if high the only choice must be to reject the product.

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Case studies involving validation/verification difficulties
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Validation or verification of manufacturing processes and associated test methods are a significant aspect of manufacture of therapeutic goods. Guidance and methodology to be used to achieve the desired validation or verification outcomes are included in regulatory guidelines. Codes of GMP, pharmacopoeias and international standards. However, such studies are not always performed properly. Three cases will be discussed where inadequate validations of microbiological quality/efficacy tests applied to therapeutic goods led to challenging outcomes for both the product manufacturers and the TGA.

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Biosecurity Risks and Biological Products
Tran Tang1
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The role of the Department of Agriculture is to maintain Australia’s favourable health status by safeguarding Australia’s environment and agriculture from the introduction of new pests and diseases from overseas. The introduction of new pests and diseases to Australia could have serious consequences for the Australian community, environment and economy. Quarantine controls at Australia’s borders minimise the risk of exotic pests and diseases entering Australia and protect our agricultural export industries as well as our environment, research capacity and lifestyle. Animal and Biological Import Assessment Branch (ABIAB) assess import permit applications for biological products, provide technical advice to regional officers, importers, general public, Ministers, and perform pre-border activities including desk audits, overseas audits, and inspections. The branch regulates while trying to facilitate the import of biological materials from live microbes to GM viral vectors. You can expect to work with our knowledgeable team anytime you bring research materials from overseas.

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Preparing for Regulatory &/or Customer Compliance Audits
Andrew Lattimore1
1. Andrew Lattimore GMP Consulting Pty Ltd, CONDER, ACT, Australia
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Back to the Future: Reinventing a New Paradigm for Risk Assessment
Paul Keese1
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From Ebola to cane toads, biological organisms continue to inflict widespread economic, social and environmental harm. This has resulted in regulatory oversight of potential weeds, pests and pathogens in a broad range of settings such as biosecurity, biocontrol agents, public health, animal and human vaccines, food contaminants, or GMOs. However, regulatory decision making has been hampered by a confusing plethora of approaches and terms used to assess and describe the risks from different types of organism. These difficulties have been compounded by the success of the chemical risk assessment paradigm, which has been widely embraced by the biological community. Nevertheless, microbiologists have long recognised the importance of certain biological/ecological characteristics that differ markedly from consideration of chemicals. In particular, there are two core elements that underpin risk assessment for any type of biological organism. These include: 1) infectivity/invasiveness, which describes the ability of an organism to spread and persist in the environment, and 2) impact, potential harm to a host that can be causally attributed to the presence of the microbe. Both of these elements are shaped by ecological interactions between a potential pathogen, its host, and the environment, including possible vectors. Therefore, risk from microbes should consider the following:
1) Infectivity/invasiveness
Organism: arrival/entry, establishment/survival, reproduction, dispersal, persistence
Host: susceptibility/resistance of the host to a microbe
2) Impact
Organism: adverse effects/virulence
Host: sensitivity/tolerance to symptoms
This presentation will describe how these key considerations elaborated by microbiologists and virologists in the 1980s and 1990s can be integrated into a common framework to assess risk across all types of regulatory regimes.
Modelling Microbial Ecology of Geobacillus spp. in Dairy Powder Plants
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Thermophilic spore-forming bacteria (TSFB) of the genera Geobacillus and Anoxybacillus can develop to high numbers on hot surfaces in milk powder processing plants where conditions are favorable for attachment, colonization and biofilm development. High spores loads in end-product develop after 16-24 hours of continuous process operation and, while not harmful to consumers, render milk powder products commercially unacceptable. Thus, the manufacturing process must be stopped periodically for cleaning, an expensive undertaking. Being able to limit thermophilic growth in milk powder processing plants promises considerable cost savings.

To enable prediction of growth of TSFB under time-varying temperature and water activity conditions relevant to powder processing plants, a mathematical model was developed from >200 growth rate determinations of 16 strains of Geobacillus spp. (The intent of the model was to identify probable ‘hot spots’ of colonization in powder plants.

A representative strain, G. stearothermophilus W14, was selected for further studies. W14 biofilm development on stainless steel surfaces exposed to flowing milk, at temperatures from 45-75°C, was assessed using a flow through device mimicking powder plant evaporator sections. Vegetative cells (VC) and spores in the milk effluent were enumerated frequently over 24h periods.

At optimal temperatures, VC initially decreased in the milk effluent, but then increased after approximately 6 hours. Spores increased after 8 hours but had counts always 2 to 3 orders of magnitude lower than VC. These time dependent changes reflect attachment, biofilm development, sporulation, and detachment processes.

The flow through system is a useful to study the growth and sporulation of thermophilic spore formers, and to explore potential interventions against spore contamination. Experiments in which temperature was cycled during the 24h processing time revealed substantially lower G. stearothermophilus spore counts for up to 24h at near optimum temperature, compared to numbers at equivalent constant temperature treatments and suggest that process run times may be able to be extended.

Duals targeting of PfA-M1 and PfA-M17: a novel mechanism for inhibition of drug resistant P. falciparum
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2. Medicinal Chemistry, Monash Institute of Pharmaceutical Sciences, Monash University, Melbourne, VIC, Australia

Plasmodium parasites, the causative agents of malaria, have developed resistance to most of our current antimalarial therapies, including artemisinin combination therapies which are widely described as our last line of defense. Antimalarial agents with a novel mode of action are urgently required. Two Plasmodium falciparum aminopeptidases, PfA-M1 and PfA-M17, play crucial roles in the erythrocytic stage of infection, and have been validated as potential antimalarial targets. Agents that inhibit the aminopeptidase enzymatic activity in parasites have been shown to control both laboratory and murine models of malaria. Similarly, the enzymes are highly conserved amongst all Plasmodium species, indicating that future therapeutics could deliver cross-species inhibition. Using compound-bound crystal structures of both enzymes, we have used a structure-guided approach to develop a novel series of inhibitors capable of potent inhibition of both PfA-M1 and PfA-M17 activity, and parasite growth in culture. Here we present our latest results on the structural and functional studies on the P. falciparum aminopeptidases.

Type 2 gingipain adhesins act in bacterial invasion
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Gingipains are multi domain peptidases that are critical virulence factors expressed by the keystone periodontal disease pathogen P. gingivalis. The adhesion regions of these proteases are multi-domain complexes that are principally comprised of a number of modules that belong to the type 1 (T1) family of gingipain adhesins (also known as cleaved adhesin domains). We have previously reported the first crystal structures of three homologous variants of these 19 kDa T1 domains and shown that they recognise a number of host target proteins. We predicted from sequence analysis and binding data that in addition to the T1 domains the adhesion regions also contain a number of other unrelated structural data on the mode of engagement of F1L with BH3-domain bearing molecules is available. We now report a crystal structure of MVA F1L in complex with a biochemically identified BH3-domain ligands. Our structure indicate that F1L engages two BH3 ligands at the same time via a canonical binding groves, analogous to the manner in which myxoma virus M17 and mammalian anti-apoptotic Bcl-x bind their respective ligands. Using structure guided mutagenesis we generated vaccinia virus bearing F1L point mutants that selectively targeted subsets of F1L BH3 domain ligands, and identified Bim as the critical pro-apoptotic effector molecule during vaccinia virus infection.
adhesins (referred to here as type 2 or T2 gingipain adhesins) and synergistically contribute to the virulence of P. gingivalis. We have recombinantly expressed and crystallized the first example of an 17.5 kDa T2 adhesin coded for by a fragment of the gene for gingipain Kgp (kpgp). We report here the structure refined at 1.05 Ångstroms resolution and thereby confirm the existence of the T2 domain family. This structure represents a new fold family which is distantly but only partially similar in topology to that of the plastocyanin/azurin family of proteins but with no copper binding sites. Like the T1 adhesins it contains a structural binding site for calcium. We observe that the recombinant T2 adhesin also binds directly to host target proteins but importantly can also inhibit bacterial invasion in cell based assays. The role of specific binding activity in the cell invasion mechanism conferred by T2 adhesins is likely to be a critical component the overall virulence of this pathogen.

Single-molecule imaging of DNA polymerase V in E. coli: mutagenesis is repressed by membrane binding and is induced by antibiotics

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Single-molecule fluorescence microscopy is allowing us to study low-abundance proteins inside live bacterial cells for the first time. We are using single-molecule imaging to study DNA polymerases in E. coli, in particular those that carry out translesion DNA synthesis on damaged DNA. When induced, TLS polymerases increase mutation rates of cells dramatically and play a demonstrated role in the development of de novo antibiotic resistance mutations. DNA polymerase V (UmuD'C) is tightly regulated in E. coli. It is produced and activated in response to DNA damage, but only after a time delay that allows error-free repair systems a chance to act first. Using single-molecule fluorescence microscopy we have discovered that pol V is also spatially regulated. We find that when first expressed, the UmuC subunit is sequestered on the inner cell membrane, repressing mutagenesis. This has led to a heightened interest in the mechanism of virulence employed by ABC toxins, coinciding with recent breakthroughs in the understanding of their structure and molecular mechanism, that in turn have largely been enabled by recent technological breakthroughs in cryo-EM.

Despite only being discovered less than 15 years ago, the ABC toxin from P. entomophaga (YenTc) has become a prototype of the TABC toxin family. It was the first ABC toxin to have its full 3D structure determined5 and remains the largest complex ever resolved (7 unique polypeptide chains arranged non-stochiometrically into a 22 subunit particle) of an ABC toxin characterised to date. More recently, we determined the structure of the 220kDa BC subunit of the YenTc using X-ray crystallography. This provided the first insights at atomic resolution into the mechanism that allows ABC toxins to in the first instance produce, store and translocate a potent cytotoxin, and subsequently release this toxin in response to specific, physiological stimuli.2 Here we will present new structural data that has allowed us to visualise at an average resolution of ~5Å the structure of the 2.1MDa YenTc BC subunit. This structure provides new insights into how the A subunit is responsible for determining host cell specificity and therefore opens up exciting possibilities for understanding differences in host susceptibility to ABC-toxin-producing pathogens, and potentially engineering new toxins for biotechnological applications.
gyrase inhibitors such as ciprofloxacin. We are developing novel flow cell devices that facilitate the rapid development of de novo resistance mutations, whilst also allowing us to monitor pol V activity. We aim to produce quantitative models that describe the contribution of pol V-induced mutagenesis to the development of antibiotic resistance.

Coordinating bacterial cell division with nutrient availability: a role for glycolysis
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Cell division in bacteria is driven by a cytoskeletal ring structure, the Z ring, composed of polymers of the tubulin-like protein FtsZ. Z ring formation must be tightly regulated to ensure faithful cell division, and several mechanisms have been described that influence the positioning and timing of Z ring assembly. Another important but as yet poorly understood aspect of cell division regulation is the need to coordinate division with cell growth and nutrient availability. In this study we demonstrate for the first time that cell division is intimately linked to central carbon metabolism in the model Gram positive bacterium Bacillus subtilis. We show that a deletion of the gene encoding pyruvate kinase (pyk), which produces pyruvate in the final reaction of glycolysis, rescues the assembly defect of a temperature sensitive ftsZ mutant and has significant effects on Z ring formation in wild-type B. subtilis cells. Addition of exogenous pyruvate restores normal division in the absence of the pyruvate kinase enzyme, implicating pyruvate as a key metabolite in the coordination of bacterial growth and division. Our results support a model in which pyruvate levels are coupled to Z ring assembly via an enzyme that actually metabolizes pyruvate, the E1 alpha subunit of pyruvate dehydrogenase. We show that this protein localizes over the nucleoid in a pyruvate-dependent manner, and may stimulate more efficient Z ring formation at the cell centre under nutrient-rich conditions when cells must divide more frequently. Ultimately this helps to ensure the survival of newborn cells.

Simulating the Influence of Diet on the Intestinal Microbiome Composition
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Diet is a driving factor in the emergence of western lifestyle disease, and of the gut microbiota composition, which has been linked to a host of diseases. ‘Diet’ comprises many dimensions: the proportion of protein, carbohydrate and fat in food; the food’s caloric concentration; the incorporation of fasting periods; and macronutrient source, such as carbohydrates in the form of sugar, complex carbohydrates or fibre. The rational design of diet interventions requires the integration of all these dimensions, which is experimentally intractable. We are developing a simulation that integrates these diet dimensions to investigate how diet drives microbiota community composition.
We simulate a community of bacteria cells and their location in the gut. We assume bacteria require access to carbon (C) and nitrogen (N) in a ratio of 5:2:1, one of which is always limiting on growth; all other nutrients are freely available. Bacteria internalise C and N from their substrates in the local environment. Internalised stores of C and N decay, representing the ‘cost of living’, and their absolute quantity determines a cell’s probability of division or death. The microbiota community composition is defined in terms of six ‘functional guilds’, based on bacteria substrates, which may be diet-derived macronutrients or mucin glycoprotein host-secretions. Fully- and partially digestion resistant carbohydrates are represented, as is casein; these are components of real diet formulations input to the simulation. The mucin secretion rate has been estimated. Guild members compete for substrates, but do not otherwise interact.
A real-world data set of 30 diets, comprising 10 macronutrient distributions and 3 energy densities, administered to 250 cages of mice is used to parameterize the simulation. The averaged food consumption of each cage is known. Analysis across these 250 simulations reveals how different diets promote or disadvantage particular guilds. We examine how the relative provision of host-secreted and diet-derived bacteria substrates, periodic fasting and the availability of aromatic fibres, both individually and in concert, shape the microbiota.

Untangling the evolutionary history of infectious diseases and the human microbiome using Neandertal dental calculus
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2. University of Aberdeen, Aberdeen, Scotland
Interpreting the evolutionary history of bacterial communities within the human body (microbiome) is key to understanding multiple aspects of health and disease, and elucidating mechanisms that underlie bacterial and human co-evolution. Although research since 1985 has suggested that the human microbiome evolved in accordance with the hominid evolutionary tree, recent evidence has indicated that the human microbiome underwent significant changes after the split between human and chimpanzee lineages. To examine this theory in greater detail, we recovered ancient bacterial DNA within dental calculus (calciﬁed dental plaque) from Neandertals, ancient and present-day humans, and greater apes to determine the evolutionary history of the hominid microbiome. Similar bacterial community structures were detected in all non-agriculturalist specimens (Neandertals, chimpanzees, African and European hunter-gatherers), revealing the existence of a once shared hominid microbiome. In contrast, a marked change was observed in the oral microbiome when humans adopted agriculture in both Europe and Africa. Significant changes in the core oral microbiome were also observed during the agricultural and industrial revolutions, as well as the modern era, revealing factors that can signiﬁcantly impact the human microbiome and alter the evolutionary signal associated between bacteria and humans. Consequently, essentially all present-day humans possess an evolutionarily recent oral microbiome that was introduced after the introduction of farming Europe, nearly 7,500 years ago. Lastly, this analysis revealed, for the first time, pathogens and diseases shared between one of our most recent hominid ancestors (Neandertals) and modern humans, revealing long evolutionary histories for several key oral and respiratory pathogens. These ancient and historical samples allow us to identify the timing of cultural and environmental events that altered the relationship between humans and their microorganisms, which may have signiﬁcant health consequences in the modern world.
Chronic Periodontitis – a destructive inflammatory disease associated with dysbiosis to produce a pathogenic polymicrobial biofilm

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Chronic periodontitis is an inflammatory disease of the supporting tissues of the teeth associated with a polymicrobial biofilm (subgingival plaque) accreted to the tooth which results in destruction of the tooth's supporting tissues. A characteristic feature of the disease-associated plaque is the emergence of proteolytic species. One of these species, Porphyromonas gingivalis has recently been described as a keystone pathogen as it dysregulates the host immune response to favour the polymicrobial biofilm disrupting homeostasis to cause dysbiosis and disease. The level of P. gingivalis in subgingival plaque above threshold levels (~10% of total bacterial cell load) has been demonstrated to predict imminent clinical attachment loss (disease progression) in humans. P. gingivalis is found as microcolonies in the superficial layers of subgingival plaque adjacent to the periodontal pocket epithelium which helps explain the strong association with underlying tissue inflammation and disease at relatively low proportions (10%) of the total bacterial cell load of the plaque. The mouse periodontitis model has been used to show that inflammation is essential to allow establishment of P. gingivalis at the levels in plaque (10% or greater of total bacterial cell load) necessary to produce dysbiosis and disease. The extracellular proteinases "gingipains" (RgpA/B and Kgp) of P. gingivalis have been implicated as major virulence factors that are critical for dysbiosis and disease. P. gingivalis and Treponema denticola are frequently found to co-exist in the pathogenic polymicrobial biofilm of deep periodontal pockets and have been co-localized to the superficial layer of subgingival plaque, suggesting interbacterial interactions. When co-inoculated intra-orally in animal models of periodontitis P. gingivalis and T. denticola exhibit a synergistic pathogenesis. The two species display a symbiotic relationship in nutrient utilization, growth promotion, motility and virulence which explains their intimate association.

The microbiome of skin and chronic wounds in type II diabetes

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Type II diabetes is a chronic health condition which is associated with skin conditions including chronic foot ulcers and an increased incidence of skin infections. The skin microbiome [microbes living on skin] is thought to play important roles in skin defense and immune functioning. Diabetes affects the skin environment, and this may perturb skin microbiome with possible implications for skin infections and wound healing. This study examines the skin and wound microbiome in type II diabetes. Ten type II diabetic subjects with chronic foot ulcers were followed over a time course of 12 weeks, sampling from both skin and wounds, with the skin microbiome compared to a control group. The diabetic skin microbiome showed differences to healthy controls, and chronic wounds were colonized by skin taxa, along with different profiles of microbes in individual patients. Differences in the skin microbiome may contribute to delayed wound healing in diabetic subjects.

Pyocyanin secreted by Pseudomonas aeruginosa inhibits growth of cystic fibrosis isolates of Staphylococcus aureus and Escherichia coli, but not Burkholderia cenocepacia

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Prevalence of key bacterial pathogens in the cystic fibrosis (CF) lung changes over time. Pseudomonas aeruginosa and Staphylococcus aureus are isolated in relatively equal proportions early in life, but P. aeruginosa becomes dominant later. To identify P. aeruginosa-secreted inhibitors, we grew strains PAO1, PA14, AES-1R(acute) and AES-1M(chronic) isogens to mid-log-phase at 37°C in Luria Broth (LB) and filtered out the cells. S. aureus, E. coli and Burkholderia cenocepacia were then inoculated into serial dilutions of the filtrate. Neat broth from PAO1, PA14 and AES-1R inhibited growth of all species. In contrast, AES1-M-filtered broth did not inhibit S. aureus or B. cenocepacia. P. aeruginosa siderophores have been shown to be inhibitory; therefore we supplemented LB with ferritin in CF-relevant concentrations to reduce siderophore load. AES-1M, with added ferritin inhibited S. aureus and B. cenocepacia. To investigate if pyocyanin was the inhibitor, a pyocyanin-negative mutant (PA14ΔphzA-G) was used and S. aureus, E. coli and B. cenocepacia grew in its neat-filtered broth. To assess strain sensitivity to pyocyanin, S. aureus, B. cenocepacia and E.coli were grown for 24hrs in the presence pyocyanin, pyocyanin+ferritin, and hydrogen peroxide, and absorbance measured over time in separate experiments. S. aureus and E. coli were inhibited by low concentrations of pyocyanin, whilst being resistant to H2O2. Presence of ferritin potentiated the effect of pyocyanin on E. coli but not S. aureus. B. cenocepacia was unaffected by 150µM pyocyanin with or without ferritin, yet inhibited by 30µM H2O2. To assess the effect of Type VI secretion (T6SS) in P. aeruginosainshibition, we assayed E. coli and B. cenocepacia growth in a PA14AT6SS growth filtrate. Both species grew, suggesting that B. cenocepacia inhibition is due to T6SS rather than pyocyanin. Results indicate pyocyanin dictates overall microbial abundance in presence of P. aeruginosina CF lung with the exception of B. cenocepacia, and CF sputum iron enhances pyocyanin expression as measured by qPCR and quantification of phenazines in P. aeruginosasupernatant.
Confirmation of a Duodenal Mucosa-Associated Microbiota

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The duodenum represents a unique niche within the digestive tract, characterised by the presence of aggressive digestive enzymes and bile. Due to the harsh conditions, the duodenum has long been considered sterile, with bacteria only present due to cross contamination or bacterial overgrowth due to immune deficiencies or motility disorders. Emerging evidence from molecular and culture based studies supports the presence of bacteria in this region in healthy individuals. However, these studies are constrained by issues of contamination during sampling, particularly from the upper gastrointestinal tract (oral cavity, saliva and stomach). In order to overcome this, we have utilised biopsy forceps protected by a sheath, allowing targeted, aseptic sampling of the duodenal mucosa. Biopsy samples were collected using the aseptic device from individuals undergoing upper gastrointestinal endoscopy with ethical approval. Following gDNA extraction, amplicon libraries spanning the V6-V8 region of the 16S rRNA gene were constructed, sequenced using the Illumina MiSeq platform, and analysed through QILIM. Microbial DNA was detected in all samples, indicating the presence of a duodenal mucosal microbiota. Sequence analysis revealed a community dominated by Streptococcus, representing up to 50% of the total bacterial load. The other most abundant genera identified were Prevotella, Lactobacillus, Veillonella, Neisseria and Porphyromonas. While these genera are typically identified in the oral microbiota, these results indicate they are also located in the duodenal mucosa. Concurrent sequencing of reagent-only controls revealed the presence of a "microbiota" in common laboratory reagents, in line with recent studies. However, there was relatively limited overlap between OTUs identified in reagents and duodenal samples (primarily Pseudomonas and Caulobacteraceae). The bacteria identified in this study therefore represent members of a unique duodenal mucosa-associated microbiota. Further investigation is ongoing into the adaption of this bacterial community to the duodenal niche and links between the duodenal microbiota and disordered gut function.

Mechanisms through which live viral vaccines generate superior long-lived humoral immunity

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The antibody response to natural infection, with a number of viruses, is life-long. Furthermore, neutralising antibody is by far the best correlate of long-term protective immunity conferred by effective vaccines. Indeed, humoral immunity to smallpox elicited by the live vaccinia virus vaccine is stable, lasts for decades, and is considered a valuable benchmark for the functional attributes of a good vaccine. We hypothesized that it is the escalating concentration of replicating virus (antigens) which provide important cues to key immune cell subsets, including CD4 T follicular helper (Th) cells and B cells in the germinal centre (GC) responses, and these are critical for induction and maintenance of robust, long-lived antibody responses. We used replication-competent or replication-poor poxviruses along with live or inactivated influenza A virus strains to infect mice. We found that replication-competent viruses, compared with poorly replicating or inactivated viruses, induced more robust Th and GC responses. These responses to replication-competent viruses persisted longer and were associated with significantly higher virus-neutralizing antibody titres. We next transferred antigen-specific B cell receptor (BCR) transgenic B cells into recipient animals and infected them with recombinant poxviruses that express a foreign antigen (recognised by the BCR) to study antigen-specific responses to infection. We found that, compared with poorly replicating viruses, replicating viruses induced increased frequency of somatic hypermutations in the variable region exon of the immunoglobulin heavy chain of transgenic B cells and this was associated with affinity maturation. Our data indicates that replicative capacity of virus directs the induction of durable, long-lived neutralizing antibody responses through generation of robust Th and GC responses.
with pairs of influenza A(H1N1)pdm09 and influenza B viruses that were circulating in humans in 2009 and 2010. Challenge outcomes varied depending on the virus combination and time-interval between primary infection and challenge. Infection with A(H1N1)pdm09 virus was observed to prevent or delay infection with an influenza B virus. Infection with an influenza B virus delayed the subsequent infection with A(H1N1)pdm09 viruses. Co-infections occurred when short periods (1 or 3 day intervals) separated infections. Ongoing shedding from the primary virus infection was associated with viral interference after the secondary challenge. Overall, these data indicate that both the time-interval between infections and the sequential combination of viruses presented are important determinants of the degree of viral interference. Influenza viruses appear to have an ordered hierarchy according to their ability to block/delay infection, which may contribute to the dominance of different viruses often seen in an influenza season. Exploiting mechanisms that induce this temporary immunity may provide novel vaccine or therapeutic strategies to overcome influenza and other respiratory diseases.

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**PATTERN OF ROTAVIRUS STRAINS CIRCULATING IN AUSTRALIA POST VACCINE INTRODUCTION**

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**Introduction.**

Rotavirus is the major cause of gastroenteritis in young children worldwide. In an effort to reduce the disease burden, two live oral rotavirus vaccines were developed. Both vaccines were introduced into the Australian National Immunisation Program in 2007. Multiple Australian studies have shown that hospitalisation for rotavirus has declined dramatically since vaccine introduction. The simultaneous introduction of two rotavirus vaccines in Australian states and territories provides a unique opportunity to compare the impact of the different vaccines on the rotavirus genotypes causing disease.

**Objective**

This study characterised the rotavirus genotypes circulating in Australia post vaccine introduction, and compared the distribution of genotypes between states using different vaccines.

**Results.**

Prior to vaccine introduction Australia-wide surveillance has shown that G1P[8] strains were the dominant type. Since vaccine introduction, the dominant type has changed yearly, with G1P[8], G2P[4] and G3P[8] representing the most dominant types between 2007-2012. G12P[8] strains have emerged as an important genotype, being first identified in 2011, and becoming a significant cause of disease during 2012, and the most prevalent genotype during 2013 and 2014. This represents the first time this genotype has been a major cause of disease in Australia.

Australia is the only country where RotaTix and RotaTeq are used in specific regions. Each year differences in genotype distribution were noted based on vaccine type.

Full genome sequence analysis of strains from the vaccine era suggests that they are genetically evolving, with changes occurring in outer capsid proteins.

**Conclusion**

This study shows the distribution of rotavirus genotypes is more diverse and dynamic since the introduction of the rotavirus vaccine program into Australia. Both rotavirus vaccines exert selective pressure on circulating strains, and in any given year the prevalent genotypes differ. However, when the entire post vaccine period is combined the overall genotype distribution appears to be similar.

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**Initiating and sustaining immune responses – lessons from infection**

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The mammalian immune system is seen as an increasingly complex network of cells and factors that orchestrate generally targeted and appropriate immune responses to limit pathology caused by viruses, bacteria and fungi. The initiation of the response that ultimately leads to the development of a protection against infection is key to our exploiting these responses to drive immunity through vaccination. Natural immunity is highly regulated and these normal immunoregulatory processes are now known to be key to the lack of potent immune responses against some cancers. The early immune activation events in experimental Salmonella infections highlight some of the key sensory pathways - the consequences of overt activation of innate and then adaptive immune responses on bacterial replication control and clearance, and the impact of early and inappropriate immunoregulation, will be discussed in the context of the experimental typhoid model.
Honing the oncolytic weapon: Increased invasiveness of vaccinia virus-infected cells
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Tumour cells possess many features which predispose them to viral infections, including an abundance of replication resources, a defective interferon response and localised evasion of host immune cells. Oncolytic viruses take advantage of these characteristics in order to preferentially replicate within tumour cells, undergoing unchecked replication and targeting cells for destruction by the host immune system. Vaccinia virus (VACV) is a well-characterised oncolytic agent, which has previously been studied in the context of its role in the smallpox vaccine. However, the new generation immunotherapeutic strain, JX-594, contains many genetic modifications which distinguish it from the parental strain. For example, a deletion of B18R raises the susceptibility of the virus to interferons, allowing healthy cells to rapidly clear infection. While various modes of delivery of JX-594 have been trialled, currently the most effective route is intratumoral injection. Unfortunately, this can hold the risk of dislodging malignant cells, allowing them to intravasate and form distal tumours. We propose that promoting VACV-induced cell invasion could improve the efficacy of intravenous delivery, and in turn decrease the risk of secondary tumour formation. Our lab has observed that many properties of infected cells favour an invasive phenotype, including high levels of cell migration, loss of cell polarity and invasion during gelatin assays. As cell invasion is often facilitated by matrix metalloproteinases (MMPs), we studied their role during infection, and found degradation of the extracellular matrix to be dependent on a number of these proteins. Furthermore, various regulators of the cytoskeleton and MMPs can be detected by qPCR and immunoblot following virus addition. This work suggests combination therapy with JX-594 and MMP inhibitors could have a synergistic effect on the treatment of invasive cancers, by limiting MMP-mediated invasion of cancer cells, and allowing VACV to target any remaining drug-resistant tumour cells.

Getting dirty - application of soil microbiology to shock and heal
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Soil contains a massive diversity of microbial species undergoing continued interactions with their environment including both abiotic and biotic. These microbes are the driving force behind soil health and are major drivers of nutrient cycling. Getting down and dirty with these microbes is producing many promising applications and highlighting the relevance of environmental microbiology to modern living.

Electric microbes are gaining much interest due to their ability to accept and donate electrons to an electrode. Electrodes can be employed to supply electrons directly to or from an environment to overcome electron donor or acceptor deficiencies. The electrodes can stimulate biological activities useful in noble metal reduction, organic compound reduction and degradation of chlorinated compounds. But to be truly useful we still require understanding of the parameters of such transfers to enable

Linking microbial community structure to functionality of a complex mesophilic dairy starter
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The community structure of a complex dairy starter culture was studied in detail. Seven distinct genetic lineages of Lactococcus lactis, one lineage of Leuconostoc mesenteroides and bacteriophages predating on the starter bacteria were found to co-exist in the functional culture (1, 2). Single colony isolates representing the genetic lineages were found to possess a variable plasmid content and phage sensitivity profiles. A beneficial effect of the bacteriophages on the compositional stability was demonstrated using multi-strain reconstituted cultures. Furthermore, the dynamics in relative abundance of specific genomie lineages of L. lactis and Lc. mesenteroides strains was monitored during cheese manufacturing (3). This allowed us to show the special functionality of each of the genetic lineages in the complex culture. Finally, long term starter culture propagation experiments will be discussed to demonstrate culture robustness in terms of culture composition and starter functionality.

us to utilize electron flow in engineering microbial communities to remediate contaminated environments. Synthetic Biology is no offering the opportunity to program these microbes to develop electrical integrated whole cell microbial biosensors for the detection of nefarious substances in real-time. Such microbes can be built to task and take advantage of the huge genetic potential around them. But to gain the most out of our experiences in the mud we have to understand the drivers of these communities, how the microbes interact individually, and how the communities interact as a whole.

Opening Pandora’s Box

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Secondary metabolite processes developed using classical strain and process engineering continue to account for the majority of the biotech market. Convoluted fermentation processes in highly complex media with highly sensitive strains are the rule rather than the exemption. Systems level analysis and design has immense potential to domesticate and refine these processes. Genomics alone has proven unhelpful and simple contrasts fail to capture the complexity of these processes. Recent advances in omics, however, enables the generation of high-density, high-quality data that are truly informative and can guide rational design. In this talk, we will demonstrate how quantitative, multi-omics studies of several clostridia and actinomycetes have been converted into operational models and used for the rational design of superior strain and processes.

The selective control of microbial growth in complex communities: from functional foods and colonic bacteria to bacteriophage and wastewater foaming

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We are interested in two applications where external agents can potentially be used to promote or inhibit the growth of microbes in complex communities. In the human colon, functional foods may alter the growth and metabolism of bacteria impacting positively on health. Three polyphenolic compounds from turmeric, known as the curcinoids, are thought to have health benefits [1] but their action on colonic bacteria is poorly understood. We have applied Liquid Chromatography Mass Spectrometry to probe these interactions using a simplified in vitro system of bacteria (Escherichia fergusonii (ATCC 35469) and Escherichia coli strains (ATCC 8739 and DH10B)) or human fecal slurries. Varying amounts of the three curcinoids were converted following 36 h of fermentation, depending on the bacterial strain and medium used and three metabolites and an additional curcumin adduct identified. This study provides insights into the bacterial metabolism of curcinoids [2] and the analytical methods developed [3] will assist future studies of the metabolic pathways for these and structurally similar polyphenols.

In wastewater treatment plants, problematic bacteria can cause foaming that reduces operational efficiencies and causes health and safety problems [4]. We are examining the potential use of bacteriophage to reduce the population of these bacteria. Our approach includes a model that pairs the dynamics of wastewater treatment processes from existing models in GPS-X software together with a newly developed add-on describing bacteriophage dynamics. The model examines the effect of bacteriophage addition on a complex wastewater treatment system and provides insights into the timeframes of bacterial population decay and need for additional phage dosing depending on phage properties [5].


Hot-wiring microbial communities for enhanced biogas production with an organic semiconductor

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With one billion tons of methane produced annually by microorganisms, biogas production can be appreciated both for its role in global organic matter turnover and as energy source for humankind. Current operations tapping unconventional gas deposits generated by microbes in coal seams or harvesting methane from anaerobic waste digestion facilities are examples where a capacity to manipulate microbial community function for enhanced biogas formation would be desirable. Here we report a 10-18 fold increase in methane production through the application of a newly discovered structure of a semiconductive crystalline form of a synthetic phenazine that manipulates the fate of reducing equivalents as a means of accelerating methane production from coal and foodwaste. We discovered that the phenazine favors acetoclastic methanogenesis by delivering electrons directly to the terminal respiratory enzyme heterodisulfide reductase. In situ amendment of coal seam associated groundwater with
the phenazine 80 m below ground level resulted in spontaneous crystal formation and a 5-10 fold increase in methane production, outperforming other enhancement methods shown in the past. Furthermore, this organic self-assembling semiconductor stimulated the biomass production of methanogenic archaea whilst the growth of sulfate-reducing bacteria was inhibited favouring methane production.

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A fast screening method for selecting Cheddar cheese flavour adjuncts based on detection of odour-active compounds

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Flavour formation in Cheddar cheese occurs mainly in the ripening stage and results from enzymatic breakdown of carbohydrates, fats and proteins by non-starter lactic acid bacteria. Cheese ripening can be a lengthy and costly process, taking up to two years for vintage Cheddar cheese. Therefore, acceleration of cheese ripening has attracted much research interest. Addition of adjutant cultures, in particular Lactobacillus, has been shown to reduce maturation time and/or improve flavour. The current study aims to develop a fast screening method in a simple cheese model to select for potential flavour adjuncts based on detection of odour-active, volatile compounds associated with desirable Cheddar cheese flavours. Candidate adjunct strains, mostly Lactobacillus, were inoculated into UHT milk at 10^0-10^1 CFU/mL in conjunction with a two-strain mix of Lactococcus lactis starter at 10^1 CFU/mL and incubated at 30°C for 7 days. On day 7, the candidate adjuncts grew to 10^3-10^4 CFU/mL, while starter counts dropped below 10^0 CFU/mL. Volatile compounds in the 7 day fermented curds were detected by headspace solid phase microextraction gas chromatography-mass spectrometry, and the flavour profiles obtained were compared to those from Cheddar cheese. Volatile compounds that are characteristic of Cheddar flavour were found, including 3-methylbutanal, diacetyl, dimethyl disulphide, acetic acid, butanoic acid, hexanoic acid and octanoic acid. The abundance of aroma compounds varied significantly by species, e.g. Lactobacillus rhamnosus and Lactobacillus helveticus strains were associated with higher concentrations of diacetyl and 3-methylbutanal respectively, while Lactococcus lactis subsp. cremoris strains produced less acetic acid and short-chain fatty acids. Promising adjunct strains will be used in pilot-scale cheesemaking and the cheese subjected to sensory analysis. Strains that produce cheese with desirable flavours, ideally also requiring shorter ripening times, will be selected for genomic sequencing to determine the genetic features and metabolic pathways associated with positive flavour properties.

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Mimivirus and Mimiviridae revive the historical debate on the true nature of viruses

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The discovery of Mimivirus (for “Mimicking Microbe” virus), a double-stranded DNA virus infecting common amoeba of the Acanthamoeba genus, followed by the analysis of its complete genome (in 2003) sent a shock wave through the community of virologists and evolutionists. By its record particle size (750 nm in diameter) and genome length (1.2 million bp), the complexity of its gene repertoire (911 protein coding genes) as well as of its particle (made of the products of more than 130 virus genes), Mimivirus blurred the established boundaries between viruses and parasitic cellular organisms. As more researchers are getting involved in the study of Mimivirus, experimental information has been accumulating on its prevalence in the environment. The Mimiviridae family is now extensively studied revealing a peculiar infectious cycle. Unique regulatory elements are governing the viral genes expression and are processed by peculiar virally encoded enzymes. The Mimiviridae cytoplasmic replicative cycle make these giant viruses the target of viral infection reactingivate the debate on viruses’ true nature. Are viruses viruses after all?

the full ‘ancestral’ state of the system defining all Apicomplexa.


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Planctomycetes - new models for microbial cells and their activities
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Planctomycetes and some related species are unique among the domain Bacteria in possessing cells with a complex plan defined by internal membranes forming separated compartments within the cell. They also possess other unique features such as cell walls composed of protein as a major polymer instead of the peptidoglycan typical of other Bacteria. All species examined display an underlying shared cell organization in which an internal intracytoplasmic membrane separates two major cell compartments, an outer ribosome-free paryphoplasm and a more central ribosome-containing pirellulosome. Some planctomycete species have three compartments, where further membranes within the pirellulosome define another compartment, the anammoxosome in anammox planctomycetes and the membrane-bounded nuclear body in Gemmata obscuriglobus. Functional features which are correlated with structural compartmentalization in planctomycetes include in G. obscuriglobus the ability to take up proteins within the paryphoplasm of the cell by a mechanism similar to receptor-mediated endocytosis of eukaryotes, and in anammox planctomycetes such as Kuenenia stuttgartiensis, anaerobic ammonium oxidation. The anammoxosome compartment is central to the biochemistry performed by anammox planctomycetes, important contributors to the global nitrogen cycle and to wastewater remediation at industrial scale. Planctomycetes will be increasingly important for progress in both fundamental and applied microbiology.

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Haloarchaea, CRISPR defence and virus attack
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The haloarchaea are a group of extremely halophilic archaea that live in salt lakes and other hypersaline environments around the world. They are known to exist with numerous viruses that can infect and kill them, and have developed elegant defence mechanisms, including CRISPR-Cas systems, in order to survive. Such systems also protect against invasion by other foreign DNAs, such as plasmids. CRISPR-Cas systems confer adaptive immunity to the cell, and act by accumulating short spacer sequences (34-39 nt) derived from invading DNAs that can be used to recognize and degrade invading nucleic acids with identical or near-identical sequence. A well-studied model haloarchaeon that can be genetically manipulated is Haloferax volcanii, originally isolated from the Dead Sea, which possesses a single CRISPR-Cas system belonging to type I-B. It has 8 Cas proteins and 3 spacer arrays. In an experimental system to study CRISPR-Cas specificity and function in this organism, it was found that Cas6B is essential for crRNA maturation but not required for the defence reaction (1). Also, six protospacer adjacent motif (PAM) sequences are recognised by the Haloferax defence system, and successful invader recognition requires a non-contiguous seed sequence of 10 base-pairs between the crRNA and the invader. We also examined the spacer sequences carried by this organism to determine if they were related to known haloviruses and plasmids, and found specific relationships to novel mobile elements and viruses (denoted Hvol-IV1 and HRV1).

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Molecular epidemiology of imported cases of leishmaniasis in Australia from 2008 to 2014
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Leishmaniasis is a vector borne disease caused by protozoa of the genus Leishmania. Human leishmaniasis is not endemic in Australia though imported cases are regularly encountered. This study aimed to provide an update on the molecular epidemiology of imported leishmaniasis in Australia. Of a total of 206 biopsies and bone marrow specimens submitted to St Vincent's Hospital Sydney for leishmaniasis diagnosis by PCR, 55 were found to be positive for Leishmania DNA. All PCR products were subjected to restriction fragment length polymorphism analysis for identification of the causative species. Five Leishmania species/species complexes were identified with Leishmania tropica being the most common (30/55). Travel or prior residence in a Leishmania endemic region was the most common route of acquisition with ~47% of patients having lived in or travelled to Afghanistan. Cutaneous leishmaniasis was the most common manifestation (94%) with only 3 cases of visceral leishmaniasis and no cases Of mucocutaneous leishmaniasis encountered. This report indicates that imported leishmaniasis is becoming increasingly common in Australia due to an increase in global travel and immigration. As such, Australian clinicians must be made aware of this trend and consider leishmaniasis in patients with suspicious symptoms and a history of travel in endemic areas.

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Isolation of thermophilic cellulolytic bacteria from sugarcane bagasse pile
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Industrial sugarcane bagasse pile, a lignocellulose-rich with relatively high temperature and anoxic interior environment, represents a good resource for the discovery of microorganisms, which produce lignocellulosic-degrading
enzymes that can function under extreme conditions comparable to those prevailing in commercial industrial processes. In the present study, cellulose-degrading activities of thermophilic anaerobic microorganisms isolated from sugarcane bagasse piles were screened for cellulase activity by culturing on carboxy-methylcellulose (CMC) agar and staining with Gram’s iodine. From a total of 502 anaerobic isolates, 121/502 (24.1%) produced halos zone on CMC agar, all of which exhibited the growth and cellulase activity at temperature range of 37°C - 60°C, and pH range of 4-11. The SB7 isolate with the maximal halos zone and the hydrolisis capacity value of 20.7 after incubation at 60°C for 48 h was further characterized and identified as Thermoanaerobacterium sp. based on 16S rRNA gene sequence similarity as well as physiological and biochemical properties. The optimal growth conditions of the SB7 isolate was at 60°C and pH 7. Maximum enzyme production (72 nmole/min/mL) was observed after 54 h. The SB7 isolate also produced xylanase (285 nmole/min/mL) and amylose (2,065 nmole/min/mL) activities, which are of value in term of industrial enzymes. Thus, the strain SB7 obtained from this study could be of interest to further characterization as a promising source for industrial applications.

Key words: Thermoanaerobacterium, cellulase, xylanase, amylose

Emerging and re-emerging viruses and their threat to human health

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Noble prize winning virologist Joshua Lederberg identified viruses as being a real threat to our existence in his quote: “the single biggest threat to man’s continued dominance on this planet is the virus.”

History suggests Lederberg was correct because there have been numerous examples of epidemics and pandemics in past centuries which have changed human history, for example epidemics of smallpox and measles in the 1500’s contributed to the decline of the Aztecs. Famously the Spanish flu pandemic of 1918-1919 killed between 40 and 50 million and infected more than a third of the world’s population, in fact WHO note that this pandemic alone killed more people in less time than any other disease before or since.

The most important emergence in recent times was undoubtedly HIV/AIDS virus which caused the largest behavioral-type pandemic in human history. The emergence of the SARS coronavirus in China highlighted two facets of globalization - how a new infectious disease which emerged in a remote area was able to spread across the planet causing 8,000 cases and 900 deaths in 30 different countries but it also demonstrated how scientific co-operation was able to control the disease spread within 8 months. The continuing spread of dengue viruses, the introduction and establishment of viruses such as West Nile, Zika and Chikungunya into new territories highlights the relative ease with which viruses can move in an age of environmental change and cheap air travel. The re-emergence of measles, a virus considered to be controlled by vaccination has highlighted holes in our control measures. Of course the recent re-emergence of Ebola in Africa has highlighted that a virus that has a high fatality rate but low infectivity can affect the world economy.

Of course scientifically and culturally we have evolved significantly since 1918, we now have vaccines and anti-viral drugs, we have improved living conditions for billions of people, more people than ever before have access to clean water, good living conditions and good health care (at least in the first world). We could be forgiven for assuming that we had infectious diseases under control, yet in the last 30 years we appear to have found ourselves in an age of emerging plagues largely of a zoonotic origin. Dealing with this emergent threat will require more than science, it will require an greater understanding of how we affect our environment, our food supply, farming practices, urbanisation, even travelling for recreation because in the 21st century we are only ever one plane ride away from an outbreak.

“The future of microbes and mankind will probably unfold as episodes of a suspense thriller that could be entitled Our wits versus their genes”
J. Lederberg. Science 2000

Automated Agar Based Diagnostic Bacteriology - morphing into One Microbiology

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Automation of Agar based Bacteriology has become a reality with a number of laboratories within Australia taking up the challenge. While most up takers aim at greater efficiency by feeding the specimen processing feature through centralisation this limits the provision of bacteriology services to the perimeter of the central laboratory and creates a microbiology void in feeding laboratories. It seems quite ironical that such an innovation could precipitate this outcome.

If one revisits the technology and open the traditional mindsets to more than just specimen processing then the most innovative and exciting aspects of this technology reveals itself in Plate reading via telebacteriology. This uses digitised images to read cultures. This transforms the way culture plates are managed into software driven image transfer process and thereby provides the opportunity to transmit the images. This opens a new and exciting frontier in the creation of the internet microbiology laboratory and provides the means to ‘decentralise’ this high value activity. At Pathology West in NSW, we are adopting this approach to expand the horizons of the central laboratory to our network of microbiology laboratories via the internet and become one microbiology laboratory spanning thousands of kilometres.

Telebacteriology provides the means to generate professional development and inclusion in the feeder laboratories instead of exclusion and de-skilling them to ‘esky packers’. The challenges rest with IT management of access and the capacity of the internet to perform the tasks in terms of bandwidth and latency. While the excitement for automation of agar processes creates greater efficiencies the innovation of telebacteriology creates new frontiers through internet bacteriology and the morphing of separate microbiology laboratories into one unified internet laboratory, spanning the perimeter of service way beyond the boundary of the central laboratory.
Vancomycin-Dependent Enterococci - An Overview
Chong Ong¹
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Vancomycin-dependent enterococci (VDE) are a subset of vancomycin-resistant enterococci (VRE) which require vancomycin for growth. Since the initial report in 1994, there have been multiple subsequent descriptions of individual clinical cases of colonization or infection caused by these organisms, as well as outbreaks. This presentation provides an overview of VDE, including clinical settings, risk factors, microbiological characteristics, mechanisms of vancomycin dependence, diagnosis and management.

Improving microbiological diagnosis of prosthetic orthopedic device infections: role of sonication
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2. Infectious Diseases, Canberra Hospital and Health Services, Garran, ACT, Australia
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Prosthetic joint replacement has become one of the most frequently performed elective surgical procedures but despite competent practices, can be complicated by failure either due to aseptic loosening or prosthetic joint infection (PJI). Current methods of laboratory diagnosis lack sensitivity and specificity with 7-39% of PJI cases being culture negative. Sonication of prosthetic components is an emerging technique aimed at optimising diagnostic sensitivity and specificity. The ongoing study is aimed at evaluating the process and role of sonication of prosthetic orthopaedic devices, despite competent practices, can be complicated by failure either due to aseptic loosening or prosthetic joint infection (PJI). Current methods of laboratory diagnosis lack sensitivity and specificity with 7-39% of PJI cases being culture negative. Sonication of prosthetic components is an emerging technique aimed at optimising diagnostic sensitivity and specificity. The ongoing study is aimed at evaluating the process and role of sonication of prosthetic orthopaedic devices, despite competent practices, can be complicated by failure.

Novel qPCR assays for the detection of causative agents of viral and bacterial meningitis
Peter Niguema¹, Simon Erskine¹, Tina Lonergan¹, Elisa Mokany¹, Caroline Furey¹, Alison Toda¹
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Meningitis is associated with high morbidity and mortality if not diagnosed in a timely manner. Diagnosis of meningitis by culture is time consuming, may lack sensitivity, requires high-level technical expertise; and in the case of bacterial infection, may return false-negative results if antimicrobial therapy has been initiated. Early identification of causative bacterial and viral agents could aid in the prompt and proper treatment of meningitis, potentially preventing adverse clinical outcomes. To this end, we are developing panels of multiplexed qPCR assays using our novel detection technology to assist with the rapid detection of 10 bacterial and viral agents associated with meningitis. Multiplexed qPCR tests have been developed using MNAzyme detection technology. In the presence of target, MNAzymes assemble from component DNA oligonucleotides. These cleave universal probes thus generating signal in real-time. MNAzyme qPCR possesses superior specificity and multiplex capacity compared to commonly used real-time chemistries. The test includes four unique panels which detect the following organisms: (1) Neisseria meningitidis, Streptococcus pneumoniae and Hemophilus influenzae, (2) Group B streptococcus, Escherichia coli and Listeria monocytogenes, (3) Herpes simplex virus type 1, Herpes simplex virus type 2 and Varicella zoster virus, and (4) Enterovirus. The multiplex panels were designed for compatibility across commonly used “open” qPCR platforms and all have been evaluated for dynamic range, analytical specificity and sensitivity and cross reactivity. The panels demonstrated robust analytical specificity for the target organisms, with no cross reactivity with other assay or panel targets, closely related organisms or organisms that generate similar symptoms. All target assays had a limit of detection of at least 50 copies per reaction as determined by Probit analysis. MNAzyme qPCR provides a flexible and unique approach to qPCR that is specific, sensitive, rapid and easily multiplexed. The assays developed are useful for rapid identification of important meningitis pathogens.

Diagnostic Microbiology in Cambodia
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The Diagnostic Microbiology Development Program (DMDP) was created in 2008 to establish microbiology laboratories in hospitals in resource-poor countries. In Cambodia, Australian and Cambodian scientists have contributed to the increase in capacity in diagnostic testing in part supported by AusAID through Volunteers for International Development from Australia (VIDA).

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DMDP collaborate with the Cambodian Ministry of Health and international partners to assist building microbiology diagnostic laboratory capacity as part of the National Strategic Plan for Medical Laboratory Services.

DMDP have supported microbiology laboratories in six Provincial Referral and National government hospitals in Cambodia. A central media-making laboratory in collaboration with the University of Health Sciences, Phnom Penh, now prepares and distributes media to the 6 facilities.

DMDP use sustainable basic microbiology diagnostic testing according to visual charts prepared by Ellen Jo Baron, Ph.D., D(ABMM), enabling identification of important pathogens such as Burkholderia pseudomallei, Streptococcus suis, Salmonella Typhi and Cryptococcus. Identification of important pathogens along with disc diffusion susceptibility testing using CLSI M02-A12 and M100-S25 has provided important surveillance data for the Ministry of Health. The author will describe findings during the 2009-2015 period, a Vibrio cholerae outbreak, 2010, and outbreaks of nosocomial infections. An antibioticogram for 2014 will be discussed. In 2014, a total of 142 (10.5%) blood stream infections were detected at six laboratory sites from 1336 patients. Burkholderia pseudomallei, 17 (12.0%) and Salmonella, 16 (11.3%) were the most common blood culture pathogens isolated. S.aureus isolates from all sites show 74% susceptibility to methicillin. 26(8%) of 345 S.aureus isolates were multi resistant MRSA. Gram negative bacilli resistance is of most concern. Blood (n=15) and urine E.coli isolates (n=46) show 33% & 30% ceftriaxone susceptibility respectively. Ceftriaxone has been heavily relied upon by Cambodian clinicians for empirical treatment.

Challenges include supply procurement, equipment maintenance, poor salaries for government staff, poor high school and university education, and language difficulties in educating lab staff and clinicians about basic clinical microbiology.

Role of Yeast 2.0 in the advancement of Synthetic Biology

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The art of turning hindsight into foresight gives the necessary insight to learn from the past, prepare for the future and conduct research using emerging sciences – such as synthetic biology – to ensure the past and the future are ever present when we frame research questions, conceptualise experiments and develop the necessary policy and regulatory frameworks. Synthetic biology is rapidly enabling the predictive engineering of complex biological systems, providing solutions to some of the most pressing challenges facing humanity and the planet this century. In 2014, the yeast Saccharomyces cerevisiae became the first eukaryote to be equipped with a fully synthetic chromosome. The global Yeast 2.0 consortium has now embarked on building the ultimate S. cerevisiae genome by 2017. If Yeast 2.0 is successfully completed, this ‘synthetic yeast’ will not just be any ordinary yeast strain. In designing the Sc2.0 strain, the natural yeast genome will be optimised by building in sites to capacitate the reshuffling of the genome at will, potentially yielding more desirable properties. With this inductible evolution system we will be able to generate millions of unique genomes that vary in architecture and gene content. Precision genome engineering technologies are steadily advancing synthetic biology into a whole new dimension of sheer possibility with significant impacts for humanity including cost-effective production of renewable biofuels and sustainable industrial chemicals; compounds for bioremediation of polluted environments; novel antibiotics, vaccines and personalised medicines; and adequate nutritious and safe food supplies. This promise, however, also poses great ethical challenges and risks. It is the responsibility of researchers, policymakers, regulatory bodies, industry, and all citizen stakeholders to engage in a meaningful dialogue about how to capitalise on the potential of synthetic biology to improve quality of life and sustain the planet while minimizing the risk for harm.

From basic biology to industrial biotechnology: Lessons learned through engineering synthetic biological networks and pathways

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Isoprenoids (a.k.a. terpenes/terpenoids) are an extremely large and diverse group of natural compounds with myriad biological functions, including roles in photosynthesis, respiration, signaling, membrane biosynthesis, and as pigments, hormones, virulence factors, vitamins, etc. Isoprenoids also have many industrial uses, ranging from specialized applications (e.g. anti-cancer and anti-malarial pharmaceuticals) through to bulk chemicals (e.g., rubbers, agricultural chemicals, fragrances, and fuel replacements). However, extracting these compounds from natural sources or chemically synthesizing them is often unfeasible. We are using metabolic engineering with systems and synthetic biology tools to re-design microbes for industrial isoprenoid production. These tools are also being applied to understand both the biological roles of isoprenoids and the regulation of carbon flux through isoprenoid pathways. Understanding flux regulation is a pre-requisite for engineering economically-competitive microbial cell factories for isoprenoid production.

Evolution of new enzyme function in bacteria: lessons from herbicide catabolism

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The acquisition of new enzyme function (neofunctionalisation) is driven by changes in selection pressures in the environment, including the introduction of new chemical challenges such as antibiotics and pesticides. Here, I will present insights into the mechanisms and constraints that control neofunctionalisation in enzymes gained from an understanding of the enzymes that comprise the atrazine catabolic pathway from Pseudomonas sp. strain ADP. In particular, I will focus on our efforts to understand how sequence\structure\function relationship govern the topography of the evolutionary landscape that dictates which evolutionary trajectories are available for the evolution of novel catalytic functionality.
Discovery and design of microbial chemoreceptors: New parts for synthetic biology

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Being able to design ligand binding proteins is a fundamental challenge in biochemistry and synthetic biology. Bacterial chemoreceptors provide remarkable examples of protein-ligand recognition: they can detect chemicals at nanomolar concentrations and they can discriminate between closely related ligands. They play a central role in chemotaxis, allowing bacteria to detect chemical gradients and bias their swimming behaviour in order to navigate towards favourable environments.

There are thousands of chemoreceptor genes in bacterial genomes, but in spite of their importance, very few of the corresponding proteins have been characterised. This represents both an unexplored source of protein-ligand pairs for decoding molecular recognition – and a vast array of ‘parts’ for use in synthetic biology.

We have begun to explore the structural and functional diversity of the chemoreceptors from Pseudomonas syringae pv. actinidiae (Psa). Psa is an important plant pathogen that is responsible for kiwifruit crop losses in New Zealand and throughout the world. Psa lives in a wide range of environments, including soil, water and plant tissues and has an unusually complex chemosensory system.

By using a new high-throughput screen, developed in my laboratory, we are characterising the complete chemosensory repertoire of Psa. Biophysical and structural characterization of the ligand binding domain (LBDs) of these receptors is providing insights into the structural basis of their molecular recognition. In addition, we are using directed evolution to generate LBDs with novel binding capabilities.

Together, these experiments are improving our understanding of how proteins selectively bind ligands, and how this process evolves. This understanding will facilitate the design of cells that can localise to a specific chemical signal, such as a pollutant or a disease marker. Ultimately, these results will pave the way for construction of advanced biological systems that incorporate multiple functions, such as cells that can sense, seek out and remediate pollutants.

Synthetic Biology Mining: Examining Community Dynamics of Mixed Species Electrogenic Biofilms

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Microbial electrolysis cells (MECs) utilise electrogenic bacteria, microbes capable of eating and breathing electrical current, for a number of beneficial applications. Fundamental to MECs are bacteria capable of utilising either an anode as an electron acceptor or a cathode as an electron donor to carry out biologically driven processes. MEC applications currently include bioremediation, biosensing, biofuel production and power generation. Although many studies have investigated the mechanisms of microbe-electrode interactions, less is known regarding the interspecies interactions within the electrogenic biofilms formed on the electrode surface. Pacing these systems in situ, such as wastewater treatment reactors, has revealed that diverse and dynamic electrode-associated microbiomes develop both spatial and temporally. Thus, it is critical to increase our understanding of microbial interactions on the electrode surface to gain a deeper understanding of the microbial community interactome in its entirety.

Microbial interactions are being studied in competitive and synergistic growth conditions to examine interactions at a cellular and molecular level. One such syntrophic interaction found in this project has been between Geobacter sulfurreducens and Pseudomonas aeruginosa, whereby the growth of the combined organisms in the presence of specific electron donor and acceptor substrates exceeds the growth of these microorganisms alone. Interestingly, this syntrophic interaction is enhanced in the presence of oxygen despite the microaerophilic nature of G. sulfurreducens. Analysis of growth dynamics, biofilm community structure, redox reaction efficiency and electrical conduction on a set matrix of electrogenic bacterial pairs will reveal novel pathways that may improve electron flow in mixed communities. The knowledge gained from this research will contribute to improved optimization of MEC power output through the application of synthetic biology techniques.

Use of yeast to screen for chemopreventatives for Alzheimer’s Disease

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Amyloid beta is a 42 amino acid peptide that is associated with Alzheimer’s Disease (AD). In its oligomeric form, the peptide kills neuronal cells, possibly commencing a process leading to the start of AD. There are considerable efforts to find ways to reduce the levels of the peptide through autophagic responses. In our studies yeast have been genetically engineered to understand how to ameliorate amyloid beta toxicity. Amyloid beta has been fused to green fluorescent protein (GFP) to enable its visualisation and oligomerisation in real time. Yeast can reasonably model aging due to the budding process. A mother yields a bud a every cell division so daughter cells comprise 50% of the population and mothers the remaining 50%. Each new bud leaves a scar that is readily visualized so the age of a mother cell can be readily ascertained. We show that the youngest cells remove the amyloid beta-GFP using autophagy, while the oldest cells retain it. The cells of intermediate age that have the fusion can be induced to degrade the fusion protein and reduce fluorescence using drugs that increase autophagy. For example, Dimebon, a drug used in AD has been shown to reduce the green fluorescence. Some compounds that inhibit oligomerisation in vitro have been shown to increase green fluorescence indicating that they are bioavailable and inhibit oligomerisation in vivo as well. The manipulated yeast offer a convenient method of in vivo screening for compounds that affect amyloid beta within cells.
Systems Biology and Ecology of Streamlined Bacterioplankton

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The salient feature of streamlined cells is their small genome size, but “streamlining” refers more generally to selection that favors minimization of cell size and complexity. The essence of streamlining theory is that selection is most efficient in organisms that have large effective population sizes, and, in nutrient-limited systems, favors cell architecture that minimizes resources required for replication. Regardless of the cause of genome reduction, lost coding potential eventually dictates loss of function, raising the questions, what genome features are expendable, and how do cells become highly successful with a minimal genomic repertoire? One consequence of reductive evolution in streamlined organisms is atypical patterns of prototrophy, for example the recent discovery of a requirement for the thiamin precursor 4-amino-5-hydroxymethyl-2-methylpyrimidine in some plankton taxa. Examples such as this fit within the framework of the Black Queen Hypothesis, which describes genome reduction that results in reliance on community goods and increased community connectivity. Other examples of genome reduction include losses of regulatory functions, or replacement with simpler regulatory systems, and increased metabolic integration. In one such case, in the order Pelagibacterales, the P system for regulating responses to N limitation has been replaced with a simpler system composed of fewer genes. Both the absence of common regulatory systems and atypical patterns of prototrophy have been linked to difficulty in culturing Pelagibacterales, lending credibility to the idea that streamlining might broadly explain the phenomenon of the uncultured microbial majority. The success of streamlined osmotrophic bacterioplankton suggests that they successfully compete for labile organic matter and capture a large share of this resource, but an alternative theory successfully compete for labile organic matter and capture a large share of this resource, but an alternative theory postulates they are not good resource competitors and instead prosper by avoiding predation. The answers to these complex questions hinge on translating gene frequencies into trait based ecological models that reflect the systems biology of cells.

Coral-associated viruses: pathogens, mutualists, and agents of evolution?

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Corals associate with diverse eukaryotic (endosymbiotic dinoflagellates of the genus Symbiodinium, other protists, fungi, endothelial algae), prokaryotic (Bacteria, Archaea), and acellular (viruses) microbiota. The cellular microorganisms associated with corals are known to confer benefits to their host by various mechanisms, including photosynthesis, nitrogen fixation, enhancing calcification and infection prevention. Conversely, under conditions of environmental stress, certain microorganisms cause coral bleaching and diseases. Stress responses and tolerance limits of corals are the result of complex interactions with its various symbionts, but the extent to which each member contributes to a particular trait is poorly understood. The viruses associated with corals have received little attention so far, and their diversity and functions in the ecology and evolution of corals are virtually unknown. In my research group, we have started to explore the diversity of coral-associated viruses in a range of coral species on the Great Barrier Reef (GBR), in healthy and diseased/bleached coral tissues, and in laboratory cultures of Symbiodinium isolated from coral. We are examining phages infecting cyanobacteria that associate with coral disease with a long-term view to develop phage therapy for disease treatment. We employ a range of methods including transmission electron microscopy, flow cytometry, PCR amplicon deep sequencing, host genome sequencing, and viral metagenomics. Our results show that the viral assemblages of corals are highly diverse; viral families are conserved among scleractinian corals from distinct geographic regions and evolutionary lineages. GBR Symbiodinium cultures have an active infection with a filamentous virus and a ssRNA virus known from free-living dinoflagellates is present in GBR corals. I will present some of the results of our work and discuss new insights gleaned into the roles that viruses play in corals.

The Rise of Citizen Oceanography: Metagenomic Advances in the Interpretation of the Marine Microbiome

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Marine microbes are the most abundant organisms in the oceans and play a critical role in fixing carbon and cycling nitrogen in nutrient cycling which serves as the bedrock to the food web. Despite this fundamental role, surveying microbial communities throughout the world’s oceans has long been an extremely expensive discipline, requiring ship time for sample collection and thereby economically constraining the number of samples collected. Large scale modeling of global biogeochemical cycles requires the collection of high-density data, both temporally and spatially in a cost-effective way. With a combination of new technologies, appropriate laboratory protocols and strategic operational partnerships we have shown that it is possible to significantly broaden our knowledge base of the ocean by engaging thousands of private ocean-going vessels that are cruising around the world’s oceans every day. This can be achieved by equipping sailing vessels with small automated sampling devices that take a variety of biological and chemical measurements. We established the basic protocol during the Indigo V Indian Ocean Concept Expedition, sailing from Cape Town to
Singapore, highlighting opportunities of this approach and developing yacht-adapted instrumentation (1).

The analysis of the metagenomic and metatranscriptomic datasets from our pilot expedition and the first wave of ‘citizen oceanographers’ has identified biogeographic patterns in microbial community composition consistent with long studied Longhurst ocean provinces, which are defined by primary productivity and thermohaline properties of ocean currents.

I will present insights gleaned from our work thus far, including mechanisms of top-down (grazing and predation) and bottom-up (resource availability) factors affecting the structure and function of microbial communities in diverse marine habitats. For example, biogeographically unique samples from within a pristine coral atoll showed an increase in the expression of genes related to photosynthesis and nutrient cycling associated with the bottom-up control of bacterial populations, but a five-fold increase in the expression of viral proteins within the lagoon, indicated a concomitant top-down control of bacterial dynamics by phages.


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Defining the differences between symbiotic and free-living cyanobacteria

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There is an increasing awareness of numerous beneficial interactions between microbes and animals. For example, gut microbes aid in digestion of complex organic matter and photosynthetic symbionts harness the power of the sun to fuel growth of corals and sponges. Sponges hosting photosynthetic symbionts represent up to 50% of the sponge community on the benthos, but can amount to nearly 90% of the communities in some tropical reefs. For several decades, eutrophication of reef environments has been linked to an increase in the heterotrophic sponge population, at the expense of phototrophic types, with impact on the nutrient balance of the ecosystem as a whole. The role the sponge microbiome plays in the fitness of holobiont in relation to environmental factors is just starting to be explored.

Our aim was to dissect out the biochemical and molecular basis of the ancient symbiosis between cyanobacteria and marine sponges of the class Demospongiae. A photosynthetic prokaryote symbiont of Phyllospongia sp. was isolated through flow cytometry sorting, the genetic material of the symbiont was then amplified and sequenced. The symbiont genome was reconstructed into contigs with a total of 2.1 Mb, comparable to the genome size of free-living marine cyanobacteria. Analysis of the symbiont genome demonstrates a near complete assembly of the defined cyanobacterial core gene set (96.5%). In comparison to the closely related free-living isolates the symbiont displays novel defence mechanisms, genes that promote/maintain interaction with the host, modified metabolic pathways as well as differences on nutrient requirements, regulation and transport capacity that reflect its adopted niche. This work provides insights into symbiosis maintenance and adaptation, and a better understanding of the genetic mechanisms underlying the biochemical fluxes between the host and symbiont.

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Biogeography of nitrogen fixers in the Australian coastal ocean

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Nitrogen fixing bacteria (diazotrophs) play a fundamental role in alleviating nitrogen limitation in oligotrophic oceanic waters. Recent evidence suggests that the marine waters of northern Australia are a potential hotspot of nitrogen (N) fixation activity, yet the microbial community underpinning this activity has not yet been explored. In addition, little information exists regarding spatial-temporal patterns of the identity and activity of diazotrophs within the largely oligotrophic marine environment surrounding Australia. To address this gap, we surveyed a number of important but distinct oceanographic provinces spanning tropical to temperate latitudes, and coupled amplicon sequencing of nifH DNA and cDNA to 15N-N-incubation experiments. Discrete diazotrophic assemblages were observed within the tropical and temperate provinces, driven by differences in the relative abundance of globally significant photoautotrophic and photoheterotrophic cyanobacteria and heterotrophic bacteria. Specifically, shifts from Trichodesmium dominated assemblages (up to 90 % of sequences) in the shallow seas of tropical northern Australia to Candidatus Alectyocyanobacterium thalassa (UCYN-A; up to 80 % of sequences) and gammaproteobacterial (up to 60 %) dominated communities in temperate systems were observed. Rates of N2 fixation were highly variable across spatial-temporal scales, ranging from < 1 to 91 nmol N L⁻¹ d⁻¹ in the tropics, from 1 to 88 nmol N L⁻¹ d⁻¹ in the subtropical Tasman Sea, and from 6 to 47 nmol N L⁻¹ d⁻¹ in temperate south Australian waters. Network analysis revealed sea surface temperature and salinity to be overarching structuring factors for diazotroph assemblages. In addition, network analysis highlighted the significant correlation between phosphate concentration and the relative abundance of taxa specific nifH transcripts, such as UCYN-A. Our findings show that Australian marine waters harbour globally significant and highly active diazotroph assemblages, and that regional physicochemical characteristics may drive the differential contribution of cyanobacterial and heterotrophic phylotypes to N2 fixation.

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Evaluation of potential phage based methods to control foam in wastewater treatment systems

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Activated sludge foaming is a worldwide problem in wastewater treatment plants that results in poor quality effluent, increased difficulty in sludge management, and increased maintenance costs. The etiological agents of
these foams are members of the Mycolata; Gram-positive, hydrophobic, filamentous bacteria embracing the genera Gordonia, Mycobacterium, Nocardia, Rhodococcus, Skermania, and Tsukamurella. No universal methods exist yet to control these foams rationally.

Fifty-three phages infective for members of the Mycolata were isolated, and fourteen then investigated for their potential use in phage therapy to control this foaming. Whole genome sequencing of these Siphoviridae phages revealed novel dsDNA sequences ranging from 14, 270 to 103, 424 kb in size and containing many novel and often unexpected genes. Nucleotide and amino acid sequence similarity levels between these phages suggested their evolutionary relatedness and revealed evidence of recombination events in their evolution. Most appeared obligately lytic and thus suitable candidates for foam bio-control.

Presence of temperate phages infecting Mycolata species was also examined as was their potential for foam bio-control following their induction in their hosts. In silico analysis of 259 Mycolata sequences revealed that 83% of Mycolata genomes contained putative genes of phage origin, and 26% appeared to possess intact and inducible temperate phages. Studies with Mycolata strains isolated from activated sludge foams supported these trends, as both spontaneous and mitomycin C mitigated prophage induction was achieved in G. alkanivorans, G. malaoae, and T. paurometabola strains. Whole genome sequencing of these suggested an ecological role for them as some encoded genes that might enhance host fitness. Should an induction agent suitable for wastewater treatment plants be identified their selective induction en masse presents a novel method of biologically controlling foams.

Expanding our view of the microbial metabolic and phylogenetic diversity through metagenomics

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Over the last decade, metagenomics has changed the face of microbial ecology. Metagenomics bypasses traditional culture-dependent approaches and holds the promise of genome-level insights into the mostly uncharted microbial world. However, for most environments it was not possible to obtain genomes from these data because the complexity of the microbial communities under consideration and limited throughput of the sequencing technology precluded assembly. Recent advances in high-throughput sequencing and development of new tools for analyzing metagenomic data are driving the evolution of this nascent field.

We are currently applying these new bioinformatics approaches to metagenomic data from a range of different habitats, which is revealing new key populations involved in methane cycling. For example, in relatively low diversity microbial communities from deep coal-associated aquifers, we have discovered a novel archaeal lineage outside of the Euryarchaeota that contains a near complete complement of genes for methanogenesis, including the methyl-coenzyme M reductase (mcr). These novel mcrA genes have previously gone undetected in nature because of their divergence to commonly used primers. Using these novel genes we could identify other divergent mcr genes in metagenomic data from several high methane flux environments, suggesting that this metabolism may be present in many other archaeal phyla. Our findings show that methane metabolism is widespread across the archaea, which has important implications for the carbon cycle and fundamentally changes our understanding of the evolutionary history of methane metabolism.

From Diagnostics to Research in Microbiology

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The primary focus of the Queensland Paediatric Infectious Diseases Laboratory is the development of rapid molecular diagnostic assays to detect and characterise infectious agents in children. This activity has resulted in the development of more than 100 molecular protocols which have been placed into routine diagnostic practice. These assays were used to characterise respiratory infections in children, which resulted in recognition that approximately 40% of these infections could not be diagnosed.

The development of these diagnostic assays, together with the characterisation of respiratory infections in children were the basis for a research program to determine if new, as yet undiscovered, viral agents were the cause of these infections. This resulted in the discovery of a new human polyomavirus from respiratory secretions taken from a child with pneumonia. Since this discovery, twelve new human polyomaviruses have been discovered, with many of these associated with the respiratory tract.

Polyomaviruses are known to cause tumours in other animals, and therefore the association of the newly discovered polyomaviruses with cancer in humans was further investigated. It was found that Merkel cell polyomavirus sequences were present in tissues from patients with non-small cell lung carcinoma, but generally not in normal control tissues taken from the same patient. The presence of virus in these tissues was confirmed by immuno-histochemical antibody staining. Additional evidence suggests that the circular viral genome is present in these tissues as a linear molecule.

Further studies are ongoing to determine if viral genome integration has occurred, and its potential effect on cell transformation. This will form the basis of future research to elucidate the putative role of MCPyV in the development of non-small cell lung carcinoma.

The exciting universe of giant viruses

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The first identified giant virus retained by the Chamberland filter, was called "Mimivirus" in 2003. With a particle of 0.7 micrometer in diameter packing a 1.2 Mb genome encoding 977 proteins, Mimivirus was the first virus overlapping the world of bacteria both in terms of particle size and genome complexity. These giant viruses are not rare and many Mimivirus relatives were then quickly isolated, culminating with Megavirus chilensis, encoding 1,120 proteins among which 7 aminoacyl-tRNA synthetases, until then considered hallmarks of cellular microorganisms. As we thought we were finally reaching the limit of viral complexity and started to build a new paradigm about the evolution of DNA viruses,
the discovery of the Pandoraviruses came ruini.ng this newly built theoretical edification. With 1.2 micron-long particles packi.ng a genome of 2.5 Mb encoding more than 2,500 proteins, Pandoravirus salinus is now surpassi.ng the complexi. ty of the smallest eukaryotic cells, such as parasitic microsporidia species. However, with less than 10% of their predicted proteins resembling anything, as well as their unique mode of replication, the Pandoraviruses clearly represent a class of giant viruses totalement unrelated to the Megaviridae. Finally, I will present the discovery of Piliovirus sibericum, isolated from a >30,000-y-old radiocarbon-dated sample of Siberian permafrost. This third type of giant virus combine an even larger pandoravirus-like particle 1.5 μm in length with a surprisingly smaller 600 kb AT-rich genome, a gene content more similar to Iridoviruses and Marsellevirus, and a fully cytoplasmic replication reminiscent of the Megaviridae. Pandoravirus-like particles may thus be associated with a variety of virus families more diverse than previously envisioned. To conclude, I will briefly present the hypotheses that have been proposed about the origin and evolution of DNA viruses and their possible link with the emergence of eukaryotes.


Predicting Long-Term Impacts of Ocean Desertification on Microbial Plankton Communities

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Climate change is causing global average sea-surface temperatures to rise, and global average ocean chlorophyll concentrations to fall. Desertification is a reference to expanding regions of low ocean productivity, which result when biological activity causes nutrient loss from warm surface waters. As with land deserts, the life forms that populate ocean deserts are adapted to extreme conditions. While the mechanisms behind ocean desertification are understood, the consequences are far from easy to predict because so little is known about most planktonic organisms and the ecological networks they form. Understanding these systems is important because of their importance to food webs, but especially because of the biological carbon pump, a process that removes large amounts of carbon from the atmosphere, sequestering it. One approach to understanding ocean warming is to study sites where ocean surface temperatures fluctuate every year in seasonal cycles, causing rhythmic transitions between cool, productive, high chlorophyll periods and ultra-low nutrient conditions. A long-term study of the Western Sargasso Sea has shown annual oscillations between a productive community dominated by eukaryotic phytoplankton in the winter and spring and a cyanobacteria-dominated community in the summer and fall. The study of microbial plankton in laboratories has revealed many extraordinary biochemical adaptations that enable cells to thrive during periods of extreme competition for nutrient resources. Throughout seasonal succession microbial plankton form interacting networks, suggesting that plankton species interact in ways that have yet to be discovered and described.

Molecular epidemiology of ESBL genes in Escherichia coli: dissemination of resistant clones and resistance plasmids

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Escherichia coli and other Enterobacteriaceae, which produce an extended-spectrum beta-lactamase (ESBL), have become the subject of many studies during recent years in both human and veterinary medicine. ESBLs confer resistance to aminopenicillins, cephalosporins (including those of the 3rd and 4th generation) and monobactams. As beta-lactams are currently the most frequently sold class of antimicrobial agents in veterinary medicine in Germany, the presence of ESBL genes limits the therapeutic options.

In E. coli, there is not a single ESBL gene but numerous ESBL genes which belong to various gene classes. Among them, ESBL genes of the CTX-M, SHV, TEM and OXA classes are most widespread. Class- or even subclass-specific PCR assays can be used for a first characterization. However, the correct identification of a specific ESBL gene requires the determination of its complete sequence from start codon to stop codon. ESBL genes can be located in the chromosomal DNA or on plasmids. Either of these locations has implications with regard to the spread of the respective ESBL gene. When located chromosomally, vertical transfer during cell division is most likely. For tracing the dissemination of such ESBL-producing isolates, various typing methods can be applied. PCR-directed phyotyping classifies E. coli isolates in different phylogroups. Multi-locus sequence typing is not suitable for short-term epidemiological analyses (e.g. outbreak investigations), but for phylogenetic analyses and long-term epidemiological studies. In contrast, pulsed-field gel electrophoresis (PFGE) is most suitable to compare isolates in short-term epidemiological studies. DNA microarrays will help to identify the virulence and resistance gene content of E. coli isolates. Next generation sequencing, followed by bioinformatic analyses, will surely be used more extensively in the future for outbreak investigations. Plasmid analysis, including S1-PFGE, restriction analysis, PCR-based replicon typing, and finally whole plasmid sequence analysis, is important to follow the dissemination of an ESBL gene-carrying plasmid between isolates, species and genera. Examples of ESBL-producing E. coli will be provided to illustrate the use of the different methods.
Using genomics to inform epidemiology

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Since 2004 technological advances have enabled us to sequence more nucleic acid and generate more data in a shorter amount of time. Decreases in cost per nucleotide sequenced, the initial price of sequencing machines and the complexity of library construction means that whole genome sequencing (WGS) is available in many research labs and an increasing number of public health microbiology labs. I will examine the use of WGS in public health microbiology, particularly the possibility of investigating organisms without culture, the interrogation of genomes where PCR may be unavailable, outbreak investigation, tracking resistance mutations and novel pathogen discovery.

Enterococcus faecium epidemiology: the changing paradigm

Sebastian van Hal

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Contents not available at time of print...

Genotyping and genome sequencing for hospital outbreak surveillance and investigation

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Genotyping tools for characterising nosocomial pathogens are becoming more discriminatory, efficient, and cost effective, enabling improved hospital surveillance, outbreak investigation, and infection control. We present the application of a molecular sub-typing based surveillance system for the detection and investigation of two different outbreaks of Methicillin-resistant Staphylococcus aureus (MRSA) in the neonatal intensive care and in surgical wards of a Sydney public tertiary hospital. The surveillance system includes the use of routine, prospective binary typing of MRSA to rapidly detect hospital outbreaks and to monitor changes in the molecular epidemiology. Whole genome sequencing (WGS) of MRSA was conducted to identify sub-clusters within the outbreaks detected. These analyses clarified possible links between cases where epidemiological information was limited, excluded cases from outbreaks, and identified genetic markers unique to particular outbreaks. The combination of binary typing and WGS has improved infection control practices, reduced nosocomial infections, and provided new insights into MRSA transmission in different settings in the hospital.

A genomic portrait of evolution and epidemic spread of a recently emerged multidrug resistant Shigella flexneri clone in China

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Shigella flexneri is the major cause of shigellosis in developing countries. A new S. flexneri serotype Xv appeared in 2000 and replaced serotype 2a as the most prevalent serotype in China. Serotype Xv is a variant of serotype X with phosphoethanolamine (PEtN) modification of its O-antigen. Frought by a plasmid encoded gene, opt, Serotype Xv isolates belong to sequence type 91 (ST91). In this study, we used illumina next generation sequencing to elucidate the genomic basis of the epidemic spread of S. flexneri serotype Xv and endemicity of shigellosis in China. A total of 59 S. flexneri isolates including representatives of 14 serotypes (1 to 4, Y, Tv, X and Xv) from different regions of China covering 10 years from 1997 to 2006 were selected for sequencing. The genomes were sequenced using Illumina 100 bp paired-end sequencing with an average of 121 fold coverage. Reads were mapped to the reference sequence 2002017 to produce 4.48 Mb per genome on average. Five published complete S. flexneri genomes were included for comparison. Whole genome sequencing of the 59 S. flexneri isolates indicated that ST91 arose around 1993 by acquiring multidrug resistance (MDR) and spread across China within a decade. Comparative analysis of chromosome and opt-carrying plasmid pSFXv_2 revealed independent origins of 3 serotype Xv clusters in China, with different divergence times. Using 18 cluster-dividing single-nucleotide polymorphism (SNPs), SNP typing divided 380 isolates from three provinces, Henan, Gansu and Anhui, into five SNP genotypes (SGs). Each province was predominated by one SG. Genomic inter-region spread of SGs was also evident. These findings suggest that MDR is the key selective pressure for the emergence of the S. flexneri epidemic clone and Shigella epidemics in China was caused by a composite of local expansion and inter-region spread of serotype Xv.

From Genomes to Communities: analyses of the polymicrobial composition in the upper respiratory tract

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Respiratory tract infections are a significant contributor to global morbidity and mortality. Consequently there are countless studies that have examined the prevalence and carriage of common respiratory pathogens such as Haemophilus influenzae, Streplococcus pneumoniae, Neisseria meningitidis, Staphylococcus aureus, rhinovirus, coronavirus, influenzae, parainfluenzae, respiratory syncytial virus and adenovirus. With vaccines being the single most effective intervention against infectious diseases it is for this reason that it is essential to understand the carriage of these pathogens in populations so that improvements in clinical care can be made through informing vaccine development as well as policies for effective implementation. The Infectious Disease Epidemiology Group, University of Southampton UK are engaged in numerous studies geared towards understanding...
these gaps in our current understanding of the carriage and prevalence of respiratory tract infectious diseases. Here we present recent findings from various projects covering the changing epidemiology of pneumococci carried in the nasopharynx amongst children after the introduction of pneumococcal conjugate vaccine and carriage of respiratory microbes within the general population. In addition we will present current research on the analysis of respiratory tract microbiomes in healthy and diseased cohorts.

Can We Predict Human Cytomegalovirus Reactivation In Naturally Infected Haematopoietic Stem Cell Transplant Recipients?

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Human cytomegalovirus (HCMV) is a herpesvirus that infects a majority of the world’s population, where it persists as a lifelong infection in a non-replicating latent state. Periodically, the virus can reactivate from latency, resulting in new infectious virus. Primary and reactivated infections are usually mild or asymptomatic in immunocompetent individuals. However, virus reactivation in immunocompromised haematopoietic stem cell transplant (HSCT) recipients occurs frequently, and this is one of the most serious post-transplant complications that can be life-threatening. Diagnosis of reactivation in HSCT patients relies upon detection of a rise in viral genome copies in blood, at which point the virus has already replicated extensively, and this can limit the effectiveness of anti-viral therapies. We utilized a high-throughput HCMV gene-specific qRT-PCR array approach to interrogate a majority of the HCMV transcriptome in allogeneic HSCT recipients with reactivated HCMV. RNA from blood drawn on a weekly basis before, during and after reactivation was screened for expression of ~130 HCMV mRNAs. This approach detected multiple viral transcripts that preceded the rise in viral genome copies by several weeks, providing evidence that patterns of viral gene transcription may serve as a useful means for early detection and/or prediction of virus reactivation. In addition to analysis of viral gene expression, we also sought to better understand why some HCMV seropositive HSCT recipients suffer from reactivation, whereas others do not. Analysis of 84 cytokine mRNAs by qRT-PCR revealed distinct expression patterns in HCMV-seropositive HSCT recipients who either did or did not undergo HCMV reactivation. For example, upregulation of IL-5, leukaemia inhibitory factor, Fas ligand and TNF superfamily member 11 was strongly associated with HCMV reactivation, whereas the parallel comparison in HCMV-seropositive recipients in which the virus did not reactivate, revealed a different subset of differentially regulated cytokines, including upregulated CCL24, IFN-γ, IL-15, IL-2 and CD40 ligand. This analysis indicates that reactivation is linked to a unique cytokine signature. Together, analysis of viral and cellular gene expression profiles in naturally infected HSCT recipients has enabled the first identification of potential novel markers and/or predictors of virus reactivation.

A tale of two signals: the divergent roles of type I interferon signalling factors in the antiviral immune response.

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Type I interferons (IFN-Is) are highly effective mediators of innate and adaptive immune responses against viruses. They mediate their wide range of effects predominantly through a canonical signalling complex, interferon-stimulated gene factor 3 (ISGF3). This complex consists of the signal transducer and activator of transcription 1 (STAT1), STAT2 and the interferon regulatory factor 9 (IRF9). In recent years it has become clear that non-canonical IFN-I signalling in the absence of individual components of the ISGF3 complex has divergent effects on the antiviral host response against viruses and the outcome of infection. We have recently shown that the three components of the ISGF3 complex play distinct roles in the antiviral immune response against lymphocytic choriomeningitis virus (LCMV). In contrast to wild type (WT) mice that clear LCMV, STAT1-deficient mice develop a lethal wasting disease following peripheral infection. The lethal disease requires STAT2- and IRF9-independent IFN-I signalling and is mediated by CD4+ T cells. By contrast, mice deficient for either STAT2 or IRF9 survive LCMV infection but are unable to clear LCMV and instead develop a chronic infection. Both, the chronic infection and lethal wasting disease are driven by dysregulated ISGF3-independent IFN-I signalling. Understanding how IFN-Is mediate their diverse effects independently of ISGF3 is a critical step in optimizing their use as therapeutic agents.

The role of Helicobacter pylori outer membrane vesicles in inflammation and immunity.

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Helicobacter pylori colonises the stomach of more than 3 billion people worldwide, resulting in a variety of symptoms including chronic inflammation, gastric ulcers and gastric cancer. Although H. pylori strains harbouring the type 4 secretion system (T4SS) are associated with more severe pathology, strains that lack the T4SS are still capable of inducing inflammation, suggesting that there are other mechanisms whereby H. pylori initiates pathology in the host. We recently identified that peptidoglycan-containing bacterial outer membrane vesicles (OMVs) produced by all H. pylori strains initiate pro-inflammatory innate and adaptive immune responses, ultimately contributing to pathology in the host. OMVs are spherical, bi-layered membrane nano-structures ranging from 20 to 300 nm in size and are produced by all Gram-negative pathogens as part of their normal growth. We reported that H. pylori-OMVs enter human epithelial cells via lipid rafts and are subsequently detected by the host pathogen recognition receptor, nucleotide oligomerization domain 1 (NOD1) that recognises Gram-negative bacterial...
peptidoglycan (PG). The recognition of PG-containing H. pylori OMVs by NOD1 resulted in the development of autophagy and pro-inflammatory IL-8 responses. Using fluorescent lifetime imaging microscopy (FLIM)-fluorescence energy transfer (FRET), we revealed that once within host epithelial cells, OMVs migrate to early endosomes where they interact with NOD1 and facilitate the development of NOD1-dependent autophagy and inflammatory responses. Moreover, we found that the degradation of OMVs via NOD1-dependent autophagy enabled the packaging of OMV proteins into host-cell derived exosomes. We identified that these OMV-containing exosomes were capable of being presented to T cells via antigen presenting cells, resulting in the generation of H. pylori-specific adaptive immune responses. Collectively, our findings identify Gram-negative bacterial OMVs as a mechanism whereby pathogens such as H. pylori initiate innate immune responses at host mucosal surfaces, and provide a method for the establishment of pathogen-specific adaptive immunity in vivo.

Improving tuberculosis control by targeting the pathogen life cycle

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Tuberculosis (TB) remains a major cause of mortality and morbidity worldwide. The current vaccine, BCG, is only partially effective against TB, and drug-resistant strains of Mycobacterium tuberculosis are emerging at an alarming rate. The development of new TB control strategies requires an understanding of how the pathogen adapts to life within the host, in particular the ability of M. tuberculosis to persist in a latent state and thus avoid immune clearance. We have shown that specific M. tuberculosis virulence factors can impact on the development of the host immune response, and deletion of such virulence-encoding genes results in highly protective TB vaccine strains. We have also used genetic screens to identify M. tuberculosis components highly expressed during infection, in order to identify potential vaccine and drug targets. When tested in animal models, novel vaccines incorporating host-expressed proteins can serve to control the growth of M. tuberculosis at different stages of the pathogen life cycle. This includes enhanced clearance of bacteria at both the active and chronic states of M. tuberculosis infection, reduced bacterial load when delivered as a post-exposure treatment, and a marked ability of these ‘multistage’ vaccines to block reactivation of disease. Our current focus is to prepare the most promising vaccines for assessment of efficacy against TB in human trials.

Respiratory syncytial virus and bacterial co-infection of the paediatric respiratory tract

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Respiratory syncytial virus (RSV) is the most significant cause of acute respiratory infection (ARI) in children. The opportunistic pathogen Streptococcus pneumoniae is commonly detected during RSV infections. However, the clinical significance of these bacterial co-infections, and their effect on the host immune system, are still being elucidated. The role of RSV/S. pneumoniae co-infection was investigated in a clinical cohort and in cell culture. Nasopharyngeal aspirates from children under two years presenting with ARI at the emergency department of the Royal Brisbane Children’s Hospital were screened for respiratory viruses and bacteria. Disease severity scores were generated upon study enrolment. All 46 infants recruited to date had viral ARI, with RSV most commonly detected (54%). S. pneumoniae was more frequently detected in RSV infections (60%) compared to other viral infections (35%). Respiratory viruses were present across the spectrum of disease severity; however, S. pneumoniae detection was significantly associated with increased severity scores, regardless of RSV presence. These results suggest that in this severely ill cohort, S. pneumoniae was the main driver of disease severity. Given the high frequency of S. pneumoniae detection in severe RSV infections, the host innate immune response to co-infection was investigated, using clinical isolates of RSV and S. pneumoniae in immortalised human bronchial epithelial cells (BEAS-2B). RSV was found to induce a type I interferon response early during co-infection, while S. pneumoniae activated NF-kB signaling. The simultaneous activation of two distinct innate immune pathways by co-infection may lead to synergistic stimulation of downstream processes, such as inflammation, perhaps contributing to enhanced morbidity. The consequences of this simultaneous activation on the host are being investigated currently. Further sample collection and investigation of immune pathways should clarify the interactions between RSV and S. pneumoniae. Understanding the roles of virus and bacteria will potentially reveal novel treatment and prevention strategies for paediatric ARI.

The role of beta- and gamma-actin isoforms in vaccinia virus actin tail morphology

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Vaccinia virus (VACV), the live vaccine used in the eradication of smallpox, is a large DNA virus that replicates in the cytoplasm of host cells. Actin is a critical component of the host cytoskeleton and also plays an important role in the replication cycle of VACV by promoting virus motility and the spread of infection. As part of its replication cycle, cell-associated enveloped vaccinia virus particles are tethered to the surface of the host cell upon exit and can be released by interactions of the viral protein A36 with a range of host proteins involved in the ARP2/3-complex-dependent actin polymerisation pathway. This results in the formation of actin ‘tails’ which can propel the extracellular enveloped virus particles to neighbouring cells. Recent developments of siRNA and isoform-specific antibodies have enabled an exploration of the functions and relative distribution of the previously poorly understood isoforms of cytoplasmic actin (CYA): beta- and gamma-actin. We have employed these techniques and more to study the role of these two CYA isoforms in vaccinia virus tail morphology and viral release, revealing divergent roles for these proteins. We have also conducted experiments to define the mechanism of this
Mutation, recombination, and selection in HIV infection.

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Human Immunodeficiency Virus (HIV) and the related monkey Simian Immunodeficiency Virus (SIV) are characterized by a high mutation rate and exist as a quasispecies within an infected host. This high mutation rate coupled with strong immune selection from the host leads to the rapid accumulation of viral “immune escape” mutations, which allow the virus to sequentially evade host immune responses.

We have studied the rates of viral mutation and selection combining bioinformatic, biostatistical, and mathematical modeling approaches with both in vitro and in vivo models of infection. Using a single cycle in vitro infection, we have shown that HIV undergoes approximately 0.4 mutation events and 14 template switching (potential recombination) events each reverse transcription cycle, and that approximately 20% of mutation events occur in association with a template switching event. In vivo, we have characterized the dynamics of immune escape during the early stages of SIV infection. We find that mutant virus often incurs a significant “fitness cost” in terms of reduced replicative capacity. The high mutation rate of the virus means that a variety of immune escape mutations are already present in the viral quasispecies before the selecting immune response arises. The dynamic interplay between virus and host immune system has important implications for vaccination, since enhanced immune pressure may in some circumstances merely increase viral evolution rates.

The Evolution of Viral Emergence

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RNA viruses are of great biological importance because of their role as agents of disease and their presumed similarity to some of the earliest replicating molecules. I will present an overview of the “rules” of evolutionary change in RNA viruses. The central concept here is that the major aspects of RNA evolution and life-history – from the way they organize their genomes to their ability to jump species boundaries – reflect an intrinsically high rate of mutation. I will also show how recent studies have revealed an amazing biodiversity of viruses in arthropods that is changing our perspective on the nature of the virosphere, as well as on viral origins and evolution. In the second part of my seminar I will examine how viruses emerge in new hosts and how virulence might evolve following a host jump. The key question here is what can we predict about viral emergence? Mathematical models suggest that when viruses like HIV or avian influenza jump into human populations, evolution in the subsequent epidemic(s) can make the disease more harmful or less harmful depending on the biological particulars. I will briefly examine the canonical case study of the evolution of virulence – the attenuation of myxoma virus following its introduction as a biological control into the European rabbit populations of Australia and Europe. I will use comparative genomics on archived isolates to determine the molecular changes that underpin the virulence evolution seen in the two epidemics, in turn making general conclusions about the evolution of pathogen emergence and virulence.

Regulated transport of the essential amino acid arginine into Toxoplasma parasites

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Apicomplexans are a phylum of obligate intracellular parasites that, by their very nature, rely on nutrient scavenging from their hosts for survival. Nutrient transporter proteins are key to nutrient scavenging, although few transporters have been characterized in these parasites. The Novel Putative Transporters (NPTs) are an apicomplexan-specific family of transporter proteins. Here, we characterize NPT1 from the apicomplexan Toxoplasma gondii (TgNPT1). Genetic disruption of TgNPT1 revealed that this transporter is essential for parasite growth and virulence. Surprisingly, we found that TgNPT1 mutants grew normally in a medium containing increased arginine levels, implicating TgNPT1 in arginine uptake. Direct measurements of arginine uptake into T. gondii parasites revealed a reduction in arginine uptake by parasites lacking TgNPT1. Expression of TgNPT1 in Xenopus laevis oocytes resulted in a marked increase in arginine uptake. These data are consistent with TgNPT1 being an arginine transporter. Expression of TgNPT1 is up-regulated in medium containing lowered arginine levels, suggesting expression of this transporter is tightly regulated to modulate arginine uptake into the parasite. Our findings highlight the importance of arginine uptake for T. gondii growth, identify the first amino acid transporter in apicomplexan parasites, and suggest that NPTs may be an apicomplexan-specific family of amino acid transporters.

Methicillin-resistant Staphylococcus aureus within Host Evolution

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Strains of Methicillin-resistant Staphylococcus aureus (MRSA) are a major cause of hospital-acquired infections worldwide and are responsible for significant morbidity and mortality; MRSA accounts for approximately 24% of S. aureus bloodstream infections in Australia. While vancomycin remains the current treatment of choice for such infections, the optimal therapy for failing patients is yet to be defined, and it is in these settings that mutations associated with drug
resistance (and other advantageous phenotypes) are likely to emerge. In order to obtain an expanded understanding of within host evolution, particularly in the context of antibiotic exposure, we have sequenced MRSA isolate series from different patients who had persistent/recurrent infections. The first series was obtained from a single patient episode, over a 77-day period, and was comprised of six ST239-MRSA blood culture isolates. The second series was obtained from multiple episodes (from a single patient), over a 19-month period, and was comprised of nine ST105-MRSA isolates associated with different infection types. Lessons learnt will be discussed in context of both evolutionary dynamism and the utility of whole genome sequencing as a diagnostic tool.

The Dynamics of Influenza A virus Reassortment
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The segmented nature of the influenza genome allows the virus to undergo reassortment (gene swapping) upon co-infection of a single cell. This process is a major contributing factor to the emergence of novel pandemic strains. In a practical context, reassortment can be utilised to produce viruses carrying the gene constellations that make them suitable for vaccine production. Despite its significance, the factors that govern gene selection during reassortment are not well understood. Using a methodology equivalent to that used for seasonal influenza vaccine seed production, we have co-infected eggs with A/Udorn/302/72 (Udorn) virus as a model seasonal strain and the egg-adapted A/Puerto Rico/8/34 (PR8) virus and tracked the genotypes throughout the reassortment process performed under the selective pressure of antibody to PR8 surface glycoproteins. In the initial stages a large variety of viruses were isolated but with subsequent rounds of growth and selection, specific gene constellations dominate. It was found that enhanced growth explained the emergence of certain gene combinations. However a few dominant viral genotypes had poor growth and we postulate that preferential packaging of gene segments drives the emergence of these viruses. Of interest, many of the final gene constellations did not maintain the three polymerase complex subunits from the same parent, despite this expectation due to their co-evolution. Overall, the PB1 and NP genes of Udorn and the PA and M genes of PR8 were found to prevail in the final viral progeny. This study of the dynamics of reassortment show it to be a largely random process initially, but selective pressures such as growth ability, gene co-selection and polymerase efficiency restrict the final viruses that dominate. Influenza virus reassortment is a complicated process and understanding the factors that govern reassortment may aid in prediction of future outbreak isolates.

Through a Mouse, Brightly: in vivo experimental evolution of the pathogenic bacterium Citrobacter rodentium
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There is a strong association between vaginal and/or amniotic fluid Ureaplasma spp. colonisation and risk of preterm birth (PTB). The novel fluoroketolide antibiotic, solithromycin (CEM-101), is highly effective against Ureaplasma spp. and evidence from pregnant sheep suggests that, unlike most macrolide antibiotics, it readily crosses the placenta. Solithromycin has two bioactive metabolites, CEM-214 and N-Acetyl-CEM-101 (N-AC-CEM-101) which have been shown in a pregnant sheep model to accumulate in the amniotic cavity following maternal administration. This has the potential to enhance the drug’s effectiveness for treatment of in utero infections. However, the antimicrobial activity of these metabolites against Ureaplasma spp. has not been established. The effects of solithromycin, CEM-214, NAC-CEM-101, and (comparison) azithromycin were tested on a collection of 100 clinical Ureaplasma spp. isolates from the United Kingdom and Australia using a modified 96-well broth microdilution method. The mean minimum inhibitory concentration (MIC) values (µg/mL) observed for the combined cohort were:

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We are locked in an arms race with a diverse army of opponents capable of evolving much faster than we are. According to World Health Organisation estimates, infectious microbes are responsible for one in four deaths worldwide. This begs the question: how do infectious microorganisms adapt to live and cause disease in their hosts, and what factors influence this arms race? We are exploiting experimental evolution to investigate pathogen adaptation using the bacterium Citrobacter rodentium with its natural host (the laboratory mouse). This model system has direct relevance to human health as C. rodentium uses the same ‘modus operandi’ as some life-threatening human Escherichia coli strains. C57BL/6 mice were orally inoculated with the bioluminescent C. rodentium derivative ICC180 and individually housed animals allowed to infect naive animals through tightly controlled mouse-to-mouse exposure, a process which was repeated weekly over a period of 6 months. Infection dynamics were followed using non-invasive real-time biophotonic imaging to identify any novel niches invaded by ‘evolving’ C. rodentium. Bacterial shedding in stools was monitored and stool and other host samples cryogenically stored each week. Despite C. rodentium’s competence as a pathogen, some “transmission failure” events were experienced, which may indicate reductive in transmissibility perhaps due to the bottlenecks inherent at each transmission step. We have identified the evolution of a hypertransmissible isolate and are currently working to identify phenotypic and genotypic changes in this ‘evolved’ C. rodentium. This work extends current flask-based experimental evolution with an evolution model which focuses on complex, medically relevant real-world environments.
solithromycin, 0.06; CEM-214, 0.29; NAC-CEM-101, 0.37; azithromycin, 1.99. One bacterial isolate showed resistance to azithromycin (MIC 16 µg/mL) and also exhibited a greater MIC against the other compounds tested. Solithromycin showed 34-fold greater activity against susceptible Ureaplasma spp. isolates than azithromycin, while CEM-214 and NAC-CEM-101 possessed approximately 22% and 17% activity of solithromycin, respectively, significantly greater than that of azithromycin. Solithromycin and its two metabolites exhibit potent activity against Ureaplasma spp. This suggests prolonged activity of the drug post-metabolism and has important implications for the treatment of infection-associated PTB.

Explosive Cell Lysis in Pseudomonas aeruginosa Biofilms

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Extracellular DNA (eDNA) is a major component of the Pseudomonas aeruginosa biofilm matrix, facilitating cell migration, adhesion and intercellular connectivity1-2. We have observed that during the early stages of development of hydrated biofilms as well as in actively expanding interstitial biofilms, that a small proportion of cells spontaneously transform into spherical cells before lysing rapidly and releasing cellular content into the extracellular milieu. We have termed this phenomenon ‘explosive cell lysis’. As this accounts for the production of all eDNA present in these biofilms as well as a number of other ‘public goods’ that benefit the biofilm community, it is important to identify the mechanism mediating this process.

We have found that exposure to exogenous agents that induce oxidative or genotoxic stress dramatically increases the frequency of explosive cell lysis. Interestingly, both internal and external stressors have been reported to stimulate expression of the pyocin gene cluster3. Pyocins are cryptic bacteriophage that function as bacteriocins and are released through cell lysis. We have found that mutants lacking the pyocin lytic endolysin (PA0629) no longer undergo explosive cell lysis, indicating that this is the mechanism responsible for this phenomenon. We have also found that explosive cell lysis in the early stages of hydrated biofilm development provides a conditioning film for bacterial attachment and microcolony formation.

In summary, we have found that explosive cell lysis can be initiated by exogenous stressors, is mediated through the pyocin endolysin PA0629 and is essential for biofilm development.


Silent Mutations in HIV-1 Reverse Transcriptase in Subtype B HIV-1 containing D67N and K70R Drug Resistance Mutations Restore Viral Fitness and Alleviate Indel Formation

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Resistance to combined antiretroviral therapy (cART) in HIV-1 infected individuals is typically due to non-synonymous
mutations that change the protein sequence; however, the selection of synonymous or ‘silent’ mutations in the HIV-1 genome with cART has been reported. These silent K65K and K66K mutations in the HIV-1 reverse transcriptase (RT) occur in over 35% of drug-experienced individuals and are highly associated with the thymidine analog mutations D67N and K70R, which confer decreased susceptibility to most HIV-1 RT inhibitor drugs. However, the basis for the selection of these silent mutations under selective drug pressure is unknown. Using Illumina next-generation sequencing, we demonstrate that D67N/K70R substitutions in HIV-1 RT increase indel frequency by 100-fold at RT codons 65-67, consequently impairing viral fitness. Introduction of either K65K or K66K into HIV-1 containing D67N/K70R reversed the error-prone DNA synthesis at codons 65-67 in RT and improved viral replication fitness but did not impact RT inhibitor drug susceptibility. The fitness advantage conferred by K65K/K66K is likely due to their ability to alleviate decreased RT efficiency on homopolymeric stretches of nucleotides and reverse the introduction of indels due to the emergence of tAMs. These data provide new mechanistic insights into the role of silent mutations selected during cART and have broader implications for the relevance of silent mutations in the evolution and fitness of RNA viruses.

Characterization of Macrolide Resistance in Nontypeable Haemophilus influenzae

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Macrolides are used to treat many respiratory bacterial infections, and the increasing prevalence of macrolide-resistant species is a significant problem. Macrolide resistance is caused by mutations in ribosomal structural elements which alter the binding affinity of macrolides to the ribosome. Resistance can also be attributed to the presence of acquired macrolide resistance genes (AMRGs), including the erm genes that code for ribosomal methylases that alter the binding affinity of the drug, and the efflux-mediating mef genes.

Macrolides are used to treat infections where nontypeable Haemophilus influenzae (NTHi) may be involved. Most NTHi isolates carry an intrinsic efflux mechanism that makes them less susceptible to macrolides than other species, but true resistance is rare and is usually attributed to ribosomal mutations. However, a recent study by Roberts et al. (2011) involving NTHi isolates derived from cystic fibrosis (CF) patients on an azithromycin placebo-controlled trial found at least one AMRG in every isolate, irrespective of the presence of phenotypic resistance. Our aim was to further investigate these AMRGs and macrolide resistance in NTHi to see if these findings could be replicated in our collection. In our study of 186 NTHi isolates from a mix of CF and non-CF patients, with and without prior macrolide exposure, we used highly specific probe-based PCR and found that none of our isolates carried any of the AMRGs found in the Roberts study (ermA, ermB, ermC, ermF, and mefA), but that false positives were frequently seen using the original PCR primers and protocol.

From our collection, only 2 were highly resistant to macrolides by Etest [azithromycin MIC>64µg/L], 5 were resistant to macrolides by Etest [azithromycin MIC>16µg/L], and 1 was susceptible to macrolides by Etest (azithromycin MIC<8µg/L). Intergeneration of whole genome sequences of these isolates revealed mutations in genes for ribosomal structural components that have previously been attributed to macrolide resistance in other species. A further 135 AMRGs were not detected in these isolates.

Discovery of a new species of mosquito specific flavivirus in Australia using an innovative sequence-independent virus detection system

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Introduction

Insect-specific flaviviruses (ISF) only infect mosquitoes but appear to regulate the transmission of pathogenic viruses by their insect host [1, 2]. Additional isolates of ISFs are required to characterise their phylogenetics, host restriction and transmission dynamics and elucidate mechanisms of interference with other viruses in the mosquito.

Methods

We screened mosquito samples from different locations in Australia for novel ISFs using an innovative, high-throughput, sequence-independent virus detection system. After inoculation of mosquito cell cultures with homogenised samples, positive-strand RNA viruses were detected in ELISA via the presence of viral dsRNA replicative intermediates in inoculated cells [3]. RNA extracted from positive cultures was tested by generic flavivirus RT-PCR to identify new ISFs [4]. Next generation sequencing, monoclonal antibodies binding assays and growth kinetics in different cell lines was undertaken to characterise these novel viruses.

Results

A new flavivirus, Bamaga virus (BgV), was isolated from Culex annulirostris collected from Cape York in 2001. Inoculation of simian and rodent cell lines revealed that vertebrate cells were poorly susceptible to BgV infection, indicative of an ISF. However, phylogenetic analysis of the RNA genome sequence of BgV grouped it with flaviviruses known to infect vertebrates, and separately from other ISF species (5). BgV was shown to be genetically and antigenically most closely related to Edge Hill, yellow fever and dengue viruses [6].

Conclusion

BgV represents a new genetic lineage of ISF that may have recently adapted to a mosquito only transmission cycle and may serve as a useful model to study the biology of ISFs, their interaction with the host mosquito and their evolution.

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**Targeting the pathogenic interactions of lyssaviruses**

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Lyssaviruses, including rabies virus and Australian bat lyssavirus are a globally distributed genus of zoonotic pathogens that cause rabies disease with a case-fatality rate in humans of 100%, resulting in over 70,000 deaths/year. In the search for new targets to combat rabies, we have undertaken a major research program to define the intracellular interface formed by lyssaviruses with the host. This research has shown that lyssaviruses form a complex array of interactions with diverse cellular factors, and clearly indicated that the viral P-protein is the major player in this interface, apparently acting as a central hub of virus-host molecular interactions. Importantly, our recent research has indicated critical roles for these interactions in the development of disease in vivo. In particular, we identified the likely site of interaction of P-protein with STAT transcription factors of the innate antiviral immune response system. Mutation of this site (a hydrophobic pocket called the W-hole) had no impact on viral genome replication, but prevented viral subversion of innate immune signalling to render virus exquisitely sensitive to interferon. Introduction of these mutations to a normally 100% lethal recombinant virus strain prevented the onset of disease in mice, identifying this site as a potential target for therapies or viral attenuation. We have now begun to exploit this new knowledge in programs aiming to develop new live attenuated vaccine strains and antiviral drugs. We are using reverse genetics/animal infection models to assess the potency and safety of modified vaccine strains, and structural biology/fragment screening methodologies to identify new compounds that may be developed toward leads in the search for novel antivirals. The presentation will introduce our new mechanistic insights into the key molecular mechanisms underlying lethal rabies disease, and current progress toward the development of new therapeutic approaches targeting these mechanisms.


**Enteroviruses Associated with Acute Flaccid Paralysis in Oceania - Results from a Decade of Surveillance**

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More than 100 human enteroviruses are classified into species A-D. Most enterovirus infections are asymptomatic but clinical manifestations can include meningitis, gastroenteritis, myocarditis and acute flaccid paralysis (AFP), a polio-like illness. The National Enterovirus Reference Laboratory, based within the Victorian Infectious Diseases Reference Laboratory, was established in 1994 and has played a major role in monitoring the polio-free status of the Oceania region including Australia, the Pacific Islands and Papua New Guinea. The laboratory routinely tests faecal specimens from cases of AFP in children less than 15 years of age for the isolation and characterisation of enteroviruses including poliovirus. From 2005 to 2014, a total of 1154 samples were received from 565 AFP cases in Oceania. Overall, 44 different serotypes of enterovirus were identified by virus isolation and/or RT-PCR from 23.7% (134/565) cases. The three most common enteroviruses associated with AFP cases in Oceania were enterovirus A71 9.0% (12/134), coxsackievirus A24 5.2% (7/134) and the newly described enterovirus C96 5.2% (7/134). Differences were noted with respect to the distribution of species A, B and C enteroviruses within Papua New Guinea, the Pacific Islands and Australia. With the introduction of direct PCR screening and sequencing methods, a number of non-cultivable enteroviruses were identified, along with seven newly described enteroviruses and a previously undescribed enterovirus.
The current on-going and hopefully now contained outbreak of Ebola virus disease (EVD) in West Africa has resulted in over 26,000 people infected and more than 11,000 deaths. An international effort has been instrumental in containing this outbreak. The return home of travellers to West Africa and infected and non-infected HCW’s taking part in the international effort has raised concern in some communities and resulted in the institution of various processes including: health questionnaires for returning travellers, health monitoring of HCW’s and in some cases home quarantine. Laboratory investigation of emerging infections that may include cultivation of the infectious agent for identification and characterisation must be performed in a high containment laboratory. In this presentation I will summarise some of the pathogens of concern and show how laboratory investigations are performed to identify the causative agent.

Current status of Ebolavirus vaccine development
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The current Ebolavirus (EBOV) outbreak in West Africa remains uncontrolled, partially because there are still no approved vaccines or therapeutics to prevent or treat these infections. In the decade prior to 2014, many EBOV vaccines candidates had been identified though none had progressed to registration for clinical use in humans. This latest and largest EBOV outbreak has renewed the urgency for an effective and safe vaccine strategy to combat further epidemics. Several EBOV vaccine candidates have now advanced to human clinical trials. These vaccines are based on their ability to elicit immune responses against EBOV glycoproteins (GPs; the major viral immunogens); DNA-based vaccines; replication-incompetent chimpanzee recombinant adenoviruses (rAds); and live-attenuated recombinant vesicular stomatitis viruses (rVSVs). This presentation will review the current status and future of EBOV vaccines.

Salmonella Typhimurium and intestinal inflammation: a pathogen centric view
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The inflammatory response to bacterial pathogens is often the result of the stimulation of pattern recognition receptors by highly conserved bacterial products collectively known as “pathogen-associated molecular patterns”. Although inflammation is most often viewed as a host defense response to combat pathogen infections, for Salmonella Typhimurium the host inflammatory response is required for its replication because essential nutrients and respiration substrates only become available in inflamed intestinal tissues. Consequently, S. Typhimurium has evolved specific adaptations to trigger inflammatory responses in the intestinal track that do not rely on the stimulation of pattern...
Bacterial chemotaxis and changing paradigms

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Bacteria have evolved to sense changes in their environment and move to change their position in order to avoid unfavourable conditions or manoeuvre towards new niches, using chemotaxis signal transduction pathways. Bacterial chemosensors respond to external stimuli with unique precision and sensitivity - a key survival trait in search for nutrients and locating a target host cell, and as such, are considered to be critical for bacterial colonisation and pathogenicity the well-researched E. coli chemotaxis system pathway (receptor-CheA/CheW-CheY-flagella) has previously served as a reference for the characterisation of chemotaxis in other bacteria. In recent years, however, our knowledge of chemotaxis pathways has progressed from a simple E. coli paradigm to a much more complex scenarios in other bacteria where similar, but more complex pathways exist. Gastrointestinal pathogen Campylobacter jejuni encodes a single chemosensory pathway relaying signal through eleven sensory receptors, seven of which sense external ligands. It also encodes other proteins involved in the chemotaxis signalling pathway that are not found in E. coli, but common in other microbes. For example: CheV, a two-domain protein consisting of fused CheW and CheY-like domains. Similarly, C. jejuni CheA is also a two-domain protein combining a catalytic HK and a regulatory RR domains, unlike the CheA in E. coli. The functions of these proteins are yet to be elucidated in C. jejuni or other bacteria. Furthermore, identification of specific chemoreceptor-ligand interactions has been problematic and C. jejuni is the only organism, other than E. coli, where specific ligands for 6 of the 7 existing external sensory receptors have been definitively identified. Notably, we have now characterised five of the seven C. jejuni external sensory receptors inactivate the aspartate chemosensory receptor, CcaA and the multi-ligand receptor CcmL, capable of responding to 5 repellents and 5 attractants. Additionally we have elucidated signalling pathway partners, particularly CheV and CheW, for each of these sensory receptors.

Acquisition of the capsule locus in Neisseria meningitidis and the loss of N-acetylgalactosamine biosynthesis

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The acquisition of virulence determinants by horizontal gene transfer (HGT) contributes to the evolution of pathogens from commensal bacterial species. Neisseria meningitidis resides in the nasopharynx of humans and causes epidemic meningitis. Typically pathogenic isolates express a capsule which is essential for invasive disease whilst non-disease isolates typically do not express a capsule. The 25 kb capsule synthesis island (cps) has been acquired by HGT and consists of a synthetic locus (region A) and associated capsule transport genes [region C] flanked by a repetitive region D and D’. Region E containing a gene of unknown function and Region B encoding capsule transport accessory proteins, CtrE and CtrF, complete the locus. Region D’ and region D contain a truncated and intact UDP-galactose epimerase. GalE, respectively. GalE is necessary for the synthesis of UDP-galactose (UDP-Gal) which is utilised in the synthesis of lipopolysaccharide. Functional archaeology of the GalE allele has revealed that there are two functional categories: a mono-functional enzyme which can only synthesise UDP-Gal, and a second bi-functional enzyme with the capacity to also synthesise UDP-galactosamine (UDP-GalNAc). Non-disease causing meningococci possessing a capsule null locus (cnl) with region E and D only, usually contain a single bi-functional GalE allele. Conversely, region D of most encapsulated meningococci contains a mono-functional galE allele that has lost the capacity to synthesise UDP-GalNAc. Region D’ is characterised by a truncated bi-functional galE2 allele. A capsule meningococcal isolate with an unusual arrangement of Region B-D only carries an intact mono-functional galE allele. These observations suggest that the meningococcal cps island is the result of two HGT events. One HGT event is the acquisition of Region B (ctrEF) downstream of region D containing a bi-functional galE in a cnl meningococcus. A second HGT cassette containing region A-C-E-D’ usually carrying a truncated bi-functional GalE allele is then acquired to create the final structure.

Vigour and Virus Hypersusceptibility: the all-natural, gym-junkie, nude mouse, plant

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Nicotiana benthamiana is used extensively in plant research, yet knowledge of its origin, genome, genetics and ecology is limited and its unparalleled susceptibility to plant viruses has, paradoxically, been correlated both directly and inversely with a disruptive 72bp insertion in its Rdr1 gene. Almost all research with N.benthamiana, including the Rdr1-insertion studies, has used the same single, widely distributed, “laboratory” strain. We identified the geographic origin of this isolate and acquired wild accessions of N.benthamiana from the extremities of its natural distribution. With one exception, wild accessions possess an insertion-free RDR1 and produce milder symptoms to a wide range of viruses. However, one wild accession has an insert-disrupted Rdr1 and displays faster virus spread and severe symptoms. A wild strain containing insertion-free Rdr1 was made increasingly virus-sensitive by increased silencing of the gene, and a lab strain intraggressed with an insertion-free Rdr1 acquired virus protection and altered RNAi characteristics. Furthermore, the integrity and expression levels of other core RNAi genes were similar across all accessions. This demonstrates that the viral
hypersensitivity, that contributes to it being popular research tool, stems from RDR1 debilitation. Analysis of the accessions, and other Australian Nicotiana family members, dates the insertion event to ~800,000ya and identifies its original genomic origin. Every genome of 30 plant species examined, and all N.benthamiana wild isolates, except those from the extreme environment of central Australia, contain an intact RDR1. This suggests that the lab strain of N.benthamiana originates from an RDR1-deficient population whose survival in the wild depends on its unusual habitat where vigour is more important than virus defence.

The Coxiella burnetii effector protein Cig57 subverts clathrin-mediated endocytosis within the human host cell

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Coxiella burnetii is an intracellular bacterium that causes the human disease Q fever. C. burnetii infects alveolar macrophages, and replicates within a spacious, lysosome-derived vacuole. This vacuole matures to feature an acidic internal environment characteristic of a lysosome. At this stage, the Dot/Icm type IV secretion system is activated and over 100 effector proteins are translocated into the host cytoplasm to modulate intracellular survival. One of the effector proteins is Cig57. Mutational analysis demonstrated that Cig57 is essential for replication of C. burnetii, however the potential properties and function of this effector have not yet been determined. Here, we have discovered that Cig57 has a role in exploiting clathrin-mediated endocytosis (CME) of the eukaryotic host. Little is known about vesicular trafficking that contributes to vacuole formation, but the clathrin-mediated pathway may aid overall success of C. burnetii inside human cells. Important endocytic sorting motifs, recognised by adapter complexes during CME, are found in Cig57. We have established that these motifs are important for normal growth of C. burnetii. Immunoprecipitations and immunofluorescence microscopy were utilised to examine the interaction and co-localisation of Cig57 to FCHO1 and FCHO2, two important proteins involved in CME. We also report that depletion of FCHO2 or clathrin, through siRNA silencing, are important for normal vacuole formation. Further, uptake of transferrin by CME is impaired in the presence of Cig57. Ultimately, these results validate the importance of Cig57 during infection, highlight a link between Cig57 and CME, and provide further insight into how C. burnetii may manipulate normal host cell function for intracellular survival.

Understanding the roles of non-coding RNAs in Enterohaemorrhagic E. coli pathogenesis

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Expression of virulence genes in pathogenic bacteria is tightly regulated in response to environmental cues at both the transcriptional and post-transcriptional level. RNAs that do not encode proteins (non-coding RNAs) are appreciated to play important roles in post-transcriptional gene regulation by interacting with mRNAs and modulating translation and stability. High throughput sequencing studies are uncovering hundreds of non-coding RNAs in pathogenic bacteria and the challenge now is to understand the function of these RNA species. A major subclass of bacterial non-coding RNA, termed small RNAs (sRNAs), requires the RNA chaperone Hfq to anneal to mRNA targets and effect regulation. Using UV-crosslinking and NextGen sequencing techniques (CRAC or CLIP-Seq) we have generated high resolution maps of Hfq-RNA interactions in the human pathogen Enterohaemorrhagic E. coli (EHEC). Within this dataset of Hfq binding sites we have identified 55 new sRNAs (Tree et al Molecular Cell 2014) and we are looking to identify the mRNA targets of these sRNAs and understand their role in pathogenesis. Recently it has been demonstrated that RNA-RNA interactions can be extracted from CRAC-Seq data allowing ncRNAs to be sequencing in complex with their mRNA targets (a technique termed CLASH). This analysis gives insights into the function of ncRNAs in vivo. Small RNAs have been shown to recruit the RNA endonuclease, RNase E, when duplexed with an mRNA target and we have recently demonstrated that sRNA-mRNA interactions can be sequenced from RNaseE CUP-Seq data. We have confirmed a subset of these interactions using translational GFP fusions. Using this dataset we have identified mRNA targets for our newly identified HEC sRNAs and have begun assigning functions to some of these novel RNA species. We have found that the EHEC specific sRNA, Ers41, represses translation of select iron uptake receptors indicating a role in modulating iron availability.

Connecting North and South: Landscape genomics of arbovirus infections of livestock in Australia

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Arbovirus infections in Australia cause significant economic impacts on livestock production and trade. Blue tongue virus (BTV), Akabane virus (AKAV) and bovine ephemeral fever virus (BEFV) are enzootic and seasonally epidemic in cattle in northern and eastern Australia. BTV is an orbivirus that is transmitted by biting midges. It occurs here as 11 serotypes distributed in two episystems, one in the far North and another in the East. AKAV, a bunyavirus also transmitted by biting midges, causes abortions in cattle, primarily at the southern extent of its distribution in NSW. BEFV is a rhabdovirus which occurs as a single serotype. Most likely transmitted by mosquitoes, BEFV causes sweeping epizootics and sporadic outbreaks across its geographic range. We have applied a combination of mass genome sequencing of virus isolates, vector population genetics and modelling of wind-borne vector displacement to analyse the structure, dynamics and evolution of these episystems. The northern BTV episystem is highly dynamic with regular introductions of new genome segment lineages from the Indonesian Archipelago and frequent segment reassortment within and between the serotypes. In contrast, the eastern BTV episystem displays limited genetic diversity, comprising
until recently only two serotypes and a single lineage of most segments. Since 2010, two additional BTV serotypes and new segment lineages have been introduced to the East. AKAV appears to show a similar pattern of dynamic reassortment with related bunyaviruses in the North and limited diversity in the East. Interestingly, although spatial modelling supports regular aerial displacement of midges from Timor, to its southern extent in the East. BEFV appears to have been introduced to Australia on only a single occasion dating back to 1956. Potential reasons for differences in the spatial dynamics of these midge-borne and mosquito-borne viruses will be discussed.

Dynamics of E.coli O157 super-shedding in a pasture-based system
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Escherichia coli O157 is a shiga toxin producing pathogen with major public health impacts. The primary reservoir of E.coli O157 is considered to be cattle. Most human disease caused by E.coli O157 is via the foodborne route, and often seen as outbreaks. In most humans, disease is characterised by diarrhea and is self-limiting. However, in some individuals (especially infants and the elderly) it can progress to haemolytic uremic syndrome (HUS) and kidney failure. In Australia in 2014 there were 0.4 cases/100,000 population of shiga toxin E.coli (STEC) infection, compared to 2.32 cases/100,000 population in the US in 2013. The health costs of STEC infections in Australia and the US are estimated to be $2.6 million and $300 million per year, respectively. To understand the dynamics of E.coli O157 shedding and super-shedding, a cohort study was undertaken in a herd of replacement dairy heifers at Camden NSW between 2012 and 2013. This cohort was sampled (fecal and rectoanal mucosal swabs) 18 times at approximately weekly intervals. The overall prevalence of E.coli O157 shedding was 44% (412/930 samples) and ranged from 10 to 94% at individual sampling points. All of the 52 heifers in this cohort yielded at least one isolate of E. coli O157 during the study period. Super-shedding (> 104 CFU / gm faeces) was detected at a sample level of 3.6% (32/893) and ranged between nil and 10%. Of the 52 heifers, 24 (46%) were detected to be super-shedding at some point during the study period. Super-shedding in a herd. Rather, all herd members of this cohort shed E.coli O157 at some point in time, and heifers experienced super-shedding events regularly and in a non-clustered pattern. Further studies on risk factors are discussed.

One health approaches to animal conservation
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One health approaches to animal conservation are becoming increasingly important as disease threatens biodiversity, ecosystem services and indirectly human health and wellbeing. The recent global spread of chytridiomycosis across different environments and into multiple species causing mass amphibian decline provides an opportunity to test the applicability of this approach (Skerratt et al 2007, Martel et al 2013). Here we discuss its successes and latest application to see whether assisted selection for disease resistance could be used as a management strategy. We found that resistance to chytridiomycosis is evolving. This is good news for the conservation of amphibians still threatened by the disease. We found greater survival in the offspring of long exposed frogs compared with those from naïve frogs as measured in laboratory transmission experiments (Bataille et al 2015). However, selection for greater survival was context specific and variable among populations. We identified MHC resistance markers which can be used to readily identify the most susceptible amphibian populations for assisted selection. Promoting innate immunity artificially may be useful in overcoming the lack of opportunities for natural selection. In addition, determining whether natural selection is occurring for other mechanisms enabling population persistence such as particular life history traits or behaviours is important. A welcome boost to this approach to managing the major biodiversity diseases, chytridiomycosis and chytridiosis is the recent publication of the genome sequence. Conservation of wildlife is the latest developments in synthetic biology and genetic engineering for human medicine.

Development and application of a bioassay for studies of Ostreid herpesvirus 1 (OsHV-1)
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Ostreid herpesvirus-1 (OsHV-1) is a pathogen of Pacific oysters (Crassostrea gigas), a high value food product and essential component of estuarine ecosystems. Since 2008, there has been a global emergence of a genotype of OsHV-1 referred to as the microvariant (μVar) that causes very high mortality disease and has seriously impacted Pacific oyster production in many countries. A bioassay is required to study this pathogen and devise disease control strategies because a permissive cell line for culture of OsHV-1 is not available. Different formats of a bioassay were evaluated whereby naïve Pacific oysters were challenged with a virus preparation to control the environment and nutrition within a physical containment laboratory. A positive bioassay result was defined by mortality of some oysters within 7 days with replication of the OsHV-1 genome demonstrated by quantitative PCR assay. Intramuscular injection of a virus
preparation into juvenile oysters provided a more sensitive test for viable OsHV-1 compared to immersion challenge of oyster spat. Challenge by immersion and cohabitation was considered more suitable for studies of transmission and pathogenesis. The bioassay was used in these different formats to determine if the age and size of oysters were independent factors affecting susceptibility to infection and disease. The bioassay demonstrated that OsHV-1 remained infectious in seawater for 48 hours and in both moist and dry oyster tissue for 7 days at 20°C. Several disinfection methods commonly used in aquatic disease control were effective for inactivation of OsHV-1 including commercial disinfectants applied according to directions (for example, Virkon-S, Du Pont; Quaternary ammonium disinfectant preparation, Livingston) and empirical doses of chemicals including sodium hypochlorite, iodine and formalin. Chlorine (used as sodium hypochlorite) at 50 parts per million available chlorine inactivated OsHV-1 in clean seawater but not in seawater after addition of protein. Chlorine at 200 ppm did not inactivate OsHV-1 in the presence of oyster tissue. This study highlighted the potential for OsHV-1 to spread on fomites and provided information that will assist in devising effective disease control strategies.

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Characterisation and comparative analyses of the Equine adenovirus 1 and 2 complete genome sequences

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Equine Adenovirus 2 (EAdV-2) causes a mild gastrointestinal disease in foals. The primary isolate of EAdV-2 was recently sequenced and the genomic organisation characterised. The results indicate that EAdV-2 does not display the characteristic Mastadenovirus genome organisation one would expect. A particularly unique feature was the identification of two E1B-55K coding regions, of which one was typical of the Mastadenoviruses. The other E1B-55K region appeared to be a fusion of a partial pIX coding sequence to an E1B-55K coding sequence more typical of the Atadenoviruses. In addition ten open reading frames ranging from 300 to 1362 bp in length were identified for which no homologue could be identified for the encoded polypeptides. The evolution of this virus appears to be unique amongst the other Mastadenoviruses including Equine adenovirus 1 (EAdV-1). In comparison to EAdV-2, the EAdV-1 genome organisation, sequenced and characterised in a previous study, was more typical of the Mastadenoviruses. The encoded EAdV-1 polypeptides had a high degree of similarity to homologues from Canine adenoviruses 1 and 2 and also Bat adenovirus TJM (B1AdV-TJM). Phylogenetic reconstructions suggested that EAdV-1 may share a common ancestor with these viruses. Collectively the results of these studies provide insight into the evolution of EAdV-1 and EAdV-2.

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Antimicrobial resistance of Escherichia coli isolated from piglets in South Eastern Australian piggeries.

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The Australian pig industry commonly uses antimicrobials for prevention of diarrhoeal diseases in neonatal and weaner piglets caused by Escherichia coli. E. coli is ubiquitous in both humans and animals. Surveillance of E. coli resistance from both healthy and diseased piglets is necessary to anticipate any potential threat to both animal and public health. The aim of this study was to assess resistance to antimicrobials used in human medicine in E. coli isolated from healthy and clinically sick piglets. A snapshot survey of 22 commercial piggeries located in South Eastern Australia: New South Wales n=9; Victoria n=10; and South Australia n=3 was conducted from September 2013 to May 2014. Faecal samples were collected from each herd (10 from pre-weaned and 40 from post-weaned piglets). Each sample was categorised according to a simple faecal consistency score (1 = firm and shaped, 2 = soft and shaped, 3 = loose, 4 = watery) and according to detection of β-haemolytic E. coli. A total of 325 E. coli isolates were tested for resistance to 27 antimicrobials using the BD Phoenix Automated Microbiology System (BD Diagnostics). The highest prevalence of resistance was to tetracycline (72%), with moderate prevalence of resistance to trimethoprim-sulfamethoxazole (45%) and chloramphenicol (37%). Resistance to cefazolin (8%), ceftiofur (6%) and colistin (3%) was less common but continued monitoring for emerging resistance to these antimicrobials is essential because of their status in human therapeutics. E. coli from Australian piglets appear to show comparable or slightly lower antibiotic resistance levels than those in European pig producing countries, however several multi-drug resistant isolates (including drugs important for human health) were observed in this study and require further investigation.

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Multi-omics of the human gut microbiome

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The performance of the human microbiome is perturbed in unknown ways by a variety of factors including disease and diet. Here we aimed to determine the impact of inflammatory bowel disease (IBD) and a resistant starch diet on the gut microbiome community composition and functional potential by application of an array of omics technologies: 16S RNA sequencing, metagenomics, metaproteomics and metabolomics. The first studies focused on a Swedish twin cohort and a longitudinal cohort for IBD. Specific bacterial species, proteins and metabolites correlated with different sub-phenotypes of the IBD: including a reduced amount of normal beneficial microbes as well as proteins involved in butyrate metabolism, suggesting potential biomarkers of IBD. For the dietary study, subjects transitioned from a baseline diet to one with low or high amounts of resistant starch. Specific bacteria, proteins...
and metabolites were shown to significantly vary in abundance when comparing baseline to the high resistant starch diet. Together these studies exemplify the use of omics to provide potential biomarkers of specific physiological states of importance to human health.

Genome dynamics and drug resistance

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Candida albicans, the most prevalent human fungal pathogen, is generally diploid but other ploidy states clearly arise and are found not only in the laboratory but also in clinical isolates. A major question motivating our work is how ploidy state and ploidy shifts affect pathogen evolution and survival, especially in responses to extreme stresses, such as exposure to antifungal drugs within the host. Survival of these stress conditions can be due to drug resistance, tolerance or persistence and we are interested in the degree to which each of these strategies is used as well as the molecular mechanisms used to achieve these different strategies. We are also particularly interested in how rapidly these different strategies can be recruited to assist in stress survival. An important clue comes from the observation that 50% of isolates that are resistant to fluconazole (FLC), the most widely used antifungal, are aneuploid and that some specific aneuploidies can confer FLC resistance. An aneuploidy the cause of resistance or does exposure to antifungals promote the appearance of aneuploidy? Our work indicates that the answer is yes: aneuploidy can be both a cause of drug resistance and a consequence of drug exposure. Furthermore, drug exposure elicits changes in cell cycle progression that lead to whole ploidy shifts. We have been following the fate of tetraploid cells as well as the evolution of diploids in the presence of drug to determine the relationship between ploidy state and shifts in ploidy state provide rapid responses to drug and how such responses affect the evolution of survival in drug stress.

Australian and international response to public health threats.

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With MERS, SARS, H1N1, H7N9 and AMR emerging globally or in our region over the last 15 years there have been more than enough public health threats to worry the public, instigate scientists, epidemiologists, infectious diseases and public health clinicians and to concern governments around the world. Ebola emerged in West Africa and presented occasionally in parts of the world where hitherto the disease has been viewed only through the lens of Hollywood. It has caused fear across the globe. MERS is now making an impact in the Republic of Korea.

This Lecture will reflect on the impact and the lessons of these public health threats from the perspective of a CMO. The importance of functioning public health systems of infection prevention and control, of One Health, of international engagement, conventions, structures and support, of clinical and scientific collaboration and of the many assets we have in Australia will be stressed.

Mechanisms underlying patterns of inverse drug resistance in the human malaria parasite Plasmodium falciparum

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The global campaign to control malaria is under serious threat from the emergence and spread of Plasmodium falciparum parasites that are resistant to the existing antimalarials. However, it is becoming increasingly evident that parasites resistant to the former ‘wonder drug’ chloroquine (CQ) exhibit hypersensitivity to a diverse range of compounds, including the frontline antimalarial lufenforine, the antiviral drug amantadine (AMT), and a number of antibiotics. Moreover, a decrease in the parasite’s susceptibility to these drugs is accompanied by a return to CQ-sensitive status. The molecular mechanism(s) underlying this pattern of inverse resistance have not been elucidated, but it is clear that mutations in the parasite’s ‘chloroquine resistance transporter’ (PfCRT) play a key role. We have established a robust system for the functional characterization of PfCRT in Xenopus laevis oocytes (Martin et al. Science, 2009), which we employed to show that a range of different CQ resistance-conferring forms of PfCRT mediate the efflux of CQ from the parasite’s digestive vacuole (i.e., away from the drug’s site of accumulation and action), whereas the wild-type protein lacks this activity (Summers et al. PNAS, 2014). Using this system, we have examined interactions between PfCRT and drugs that possess activities that are inversely-correlated with that of CQ. Our data indicate that there are at least two different mechanisms by which PfCRT can increase the potency of certain drugs against CQ-resistant parasites. This work, and the potential for designing combination therapies that pair antimalarials with opposing selection forces, will be discussed.

Bacterial antimicrobial resistance – how compounds are extruded from cells by secondary multidrug transporters

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Bacterial multidrug resistance poses an ever increasing threat to human health. Integral to this are multidrug resistance transporters, which are membrane proteins that have the ability to expel a broad spectrum of chemically dissimilar drugs out of the cell in an energy dependent manner, using either ATP hydrolysis or the ion motive force. These efflux proteins fall into one of five families: Major Facilitator Superfamily (MFS), ATP binding cassette (ABC), Resistance-Modification-Division (RND), Small Multidrug Resistance (SMR) and Multidrug And Toxic compound Exporters (MATE). Structural information on these classes of proteins is limited due to problems inherent to isolating and crystallising integral membrane proteins. Thus, we have combined bioinformatics, genetics, biophysical, and biochemical tools to examine the multidrug resistance capacity of the QacA efflux protein from Staphylococcus aureus. QacA is a MFS family membrane protein, containing 14 transmembrane segments (TMS), that confers resistance to more than 30 different cationic, lipophilic antimicrobial compounds commonly used as antiseptics and disinfectants.
Comparison of structures and the efflux mechanism of QacA with the 12 TMS MdfA MFS multidrug resistance protein from Escherichia coli has provided insight into how secondary transporters deal with compounds of different valencies. Additionally, construction and analysis of mutants, including chimeras between these two proteins, has identified a core 12 TMS structure required for active transport by both proteins.

Antimicrobial resistance as a disaster risk problem - insights from an outsiders perspective

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The rise of antibiotic resistance is leading to a future of untreatable bacterial infections and with that, the possibility of untreatable bacterial infectious outbreaks, epidemics and pandemics. Antibiotics are one of the tools that we currently have to treat such outbreaks so with their effectiveness declining, the impact of such infectious outbreaks would become more severe. With few new drugs entering the market, it’s imperative that we use our current stocks of antibiotics wisely. This is simply because more antibiotic use leads to more antibiotic resistance. Despite the finest efforts by medical and health policy makers and communities to control the antibiotic resistance problem, the issue has reached a critical tipping point and calls for more to be done have been voiced by respected organisations. These calls include those from the WHO and CDC and respected individuals such as Chief UK Science Officer, Professor Dame Sally Davies, who has suggested antibiotic resistance be placed above the issue of terrorism on national risk registers. Increasingly, the language surrounding antibiotic resistance is reminiscent of that used for natural disasters and it is clear that the issue is no longer a “business as usual problem”. Given the current state of the antibiotic resistance problem and the likely near future of untreatable bacterial infections, this talk suggests an alternative and novel policy perspective. With this in mind, this talk proposes to make antibiotic resistance a broader issue and to rebrand it as a disaster risk problem and to engage the expertise of emergency managers. When we think of natural disasters, we often think of tsunami, wild fires or floods etc, however, infectious diseases are just as much a risk to communities as any other natural disaster. Governments and disaster policy makers across the world use the Emergency Risk Management process in the management of and preparation for natural disasters. This process is a systematic method that through engagement with multiple stakeholders, identifies, analyses, evaluates and treats risks and takes an iterative approach with well-defined activities that lead to implementation of risk-treatment strategies tailored to a specific community’s risk profile. This policy and practice framework is an excellent mechanism for reaching out to communities and communicating complex messages. Rebranding antibiotic resistance as a disaster risk problem has the following benefits:

1. The Community understands disasters. A rebranding of the issue will give the antibiotic resistance issue the weight it deserves.
2. The disaster risk framework will provide new ideas and innovations in dealing with the antibiotic resistance problem (that would be discussed in our article).
3. The framework has been successfully used to communicate and prepare communities for natural disasters. These channels will be of great benefit to communicating the gravity of antibiotic resistance and associated risk mitigation strategies to the Community.

Why are new antibiotics so hard to find?

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The antibiotic pipeline is broken, with a dearth of new antibiotics, a collapse in pharmaceutical company research, and the exhaustion of chemical diversity contained in pharma libraries. “The low hanging fruit from the antibiotic tree has probably already been picked and new sources of compounds are needed.”2 This talk will illustrate three different approaches we have been investigating to uncover and develop new antibiotics:

1) In order to discover new therapies, the rational evolution of existing antibiotics has proven to be more effective than screening for new structures. We postulated that membrane-targeting motifs could be appended to drugs acting on membrane-anchored targets, enhancing membrane binding and concomitantly increasing drug concentration at the target site. We selected vancomycin to test this approach, as this prototypical glycopeptide inhibits peptidoglycan formation by binding membrane-bound lipid II. An comprehensive medicinal chemistry campaign has prepared over 300 analogues, leading to promising candidates that have been extensively characterised.

2) During the ‘golden age’ of antibiotic discovery, many potentially promising compounds were discovered but never developed. We have been investigating the octapeptins, naturally derived products similar to the commonly prescribed polymyxin antibiotics. The octapeptins show promising broad-spectrum activity against Gram-negative bacteria, most importantly against polymyxin-resistant species.

3) Antibacterial drugs occupy a unique property space that is vastly different to drugs developed for other therapeutic areas, suggesting that commercially available chemical compounds designed for ‘druglike’ properties lack the physicochemical properties ideal for activity against bacteria and therefore, alternate sources of chemical diversity need to be investigated. We believe that there is an untapped resource contained in the laboratories of synthetic organic chemists; compounds prepared for other projects that have never been tested for their antimicrobial potential. We have created a Wellcome Trust-supported not-for-profit open-access pipeline, The Community for Open Antimicrobial Drug Discovery (CO-ADD) as a global screening initiative to uncover this significant and rich chemical diversity, providing unencumbered free antimicrobial screening for any interested researcher.

Cathelicidins in the Tasmanian devil (Sarcophilus harrisii)

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The Tasmanian devil (Sarcophilus harrisii) is the largest remaining carnivorous marsupial. The future of the Tasmanian devil is under threat from a contagious cancer, Devil Facial Tumour Disease (DFTD), which has already decimated over 85% of the population. Interestingly, young devils do not catch DFTD, and we hypothesize that antimicrobial peptides within the mother’s milk may protect the young against infection. Cathelicidins are a family of antimicrobial peptides within mammals which contribute to innate immunity through antimicrobial and immunomodulatory functions. They have been studied extensively in eutherian mammals but marsupials are relatively unexplored. Marsupials have a short gestation period and give birth to altricial young which are immunologically naive. During development the young are protected from microbial infection by cathelicidins expressed in the mother’s milk, pouch epithelium and skin of the young themselves. This unique reproductive physiology has encouraged lineage specific expansion of the cathelicidin gene family within marsupials, resulting in numerous diverse peptides. We identified six cathelicidins in the Tasmanian devil genome and have synthesized the mature peptides. These will be tested against a range of pathogens, including DFTD cells. Thus far only bacteria and fungi have been tested, with promising results. One cathelicidin is capable of killing Cryptococcus gattii and Cryptococcus neoformans, but is not effective against human cells. The aim of this study is to test cathelicidins in the Tasmanian devil to provide new drugs to treat human and animal disease.

Novel antimicrobial therapy that targets selenium metabolism to treat Clostridium difficile infection.

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Clostridium difficile infection (CDI) is responsible for 250,000 infections and 14,000 deaths annually. High rates of treatment failure, recurrent infections, and potential for antibiotic resistance are all evidence that new and effective treatments for CDI are needed. A potential treatment for CDI is Auranofin, which disrupts the selenoproteins used for anaerobic metabolism in C. difficile. The aim of this study is to assess the potential of Auranofin as a treatment for CDI. The effect of Auranofin on C. difficile growth was tested on CD01 (ribotype UKI-001) which is the most commonly isolated ribotype in Australia. Growth assays using optical density and viable cell counts showed that Auranofin inhibits the growth of C. difficile at concentration of 50µM when compared to the control (p<0.0001). No viable cells were recovered from the Auranofin treated cultures, indicating that Auranofin is bactericidal against C. difficile. Sporulation assays indicated that Auranofin also inhibits sporulation, with no viable spores recovered from the Auranofin treated cultures. Cell cytotoxicity assays showed that Auranofin treated cultures had less cytotoxic activity against vero cells than the no treatment control (p<0.001) but is not significantly different to the ethanol control. Growth assays comparing Auranofin to Metronidazole, one of the current treatments for CDI, were also performed. These assays indicated that there is no significant difference between the two treatments when it comes to inhibiting C. difficile growth, cell viability or sporulation. The bactericidal activity, pharmacokinetics and prevention of sporulation make Auranofin a good candidate for a CDI treatment. Auranofin may also be narrow spectrum, with just 14% of bacteria utilising selenoproteins. Further experiments will test Auranofin in a CDI and recurrent CDI mouse model. The aims of these experiments will focus on treatment of disease, prevention of recurrent disease, disease severity and effects on the intestinal microbiota.

Kangaroos and cattle: anaerobic fermentation with and without methane production

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Cattle and kangaroos are both grazing herbivores that have developed an enlarged forestomach (the rumen in cattle), whereby anaerobic fermentation of plant material by a complex microbial consortium takes place prior to gastric digestion. During this process hydrogen is produced and must be removed. In cattle, this is done predominantly by the action of Archaea and the production of methane. Indeed, 10 - 14% of Australia’s total greenhouse gas emissions are from enteric methane emitted from ruminants. However, this is not the case with kangaroos, which emit considerably less methane, particularly from forestomach fermentation. Early studies indicated that methanogens were often below detectable levels in kangaroos but reductive acetogens were always detectable, suggesting that the dominant mechanism in kangaroo forestomach may be reductive acetogenesis, undertaken by a diversity of bacterial species. With the use of targeted isolation, PCR and gene sequencing it was shown that the Archaea present in kangaroos were very different to those in ruminants and may even include methanotrophic Archaea. Reductive acetogens were isolated that were phylogenetically distinct from previously cultivated species and were present in the 42 kangaroos examined. While good evidence for the dominance of reductive acetogenesis, this was still circumstantial. By using heavy carbon (13C) tracing, it has been possible to show that in rumen contents incubated with heavy CO2 and H2, 13CH4 is rapidly produced but labelled acetate was absent, whereas the same incubation with kangaroo foregut contents resulted in heavily labelled acetate but little labelled methane, confirming the dominance of reductive acetogenesis in the kangaroo foregut. Further knowledge of the ecosystem that supports reductive acetogenesis is being compiled using comparative amplicon sequencing, metagenomics and metatranscriptomics. Both foregut microbial ecosystems are healthy, naturally occurring, ecosystems that function in a similar manner but with fundamentally different hydrogen utilising communities.
Degradation of insensitive munition ingredient 2,4-dinitroanisole by bacteria
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2,4-Dinitroanisole (DNAN) is an insensitive munition ingredient used in explosive formulations as a replacement for 2,4,6-trinitrotoluene (TNT). Little is known about the environmental fate of DNAN. We have recently isolated a Nocardioides sp. strain JS1661 which has an ability to grow on DNAN as a sole source of carbon and energy. Identification of the genes encoding the key enzymes suggested recent evolution of the pathway by recruitment of a novel hydrolase to extend the well-characterized 2,4-DNP pathway. Potential of JS1661 and its novel hydrolase for waste treatment and explosive degradation is also discussed.

Impacts of climate change factors on eucalypt ectomycorrhizal fungi
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Soil fungi play important roles in forest carbon and nutrient cycles, but relatively little is known about how they will respond to future climate change, especially in the context of southern hemisphere forest ecosystems. Our recent research focus has been to use controlled environment glasshouse and microcosm experiments to investigate the interactive effects of elevated atmospheric CO₂, temperature and drought on Australian forest soil fungi, including those that form ectomycorrhizal associations with eucalypts.

In a glasshouse experiment, Eucalyptus saligna and E. sideroxylon seedlings were grown in field soil and maintained for 5 months under sub-ambient (280 ppm), ambient (380 ppm) and elevated (640 ppm) atmospheric CO₂ conditions at both 26°C and 30°C. Multivariate analyses of molecular data showed a significant (P < 0.035) separation between fungal communities associated with the two different tree species and a clear separation between the communities from the 280, 400 and 640 ppm CO₂ treatments at 34°C. This response appeared to be plant-dependent at 280 and 400 ppm CO₂, however, all 640 ppm CO₂ samples clustered together regardless of tree species. Interestingly, several of the key fungal species identified to respond strongly to the climate change factors were ectomycorrhizal fungi, including Pisolithus sp. so we performed a subsequent microcosm experiment and used transcriptomics to investigate the response of E. grandis (for which a genome sequence is available) to colonization by different Pisolithus isolates under ambient (400 ppm) and elevated (650 ppm) CO₂. Our data showed that E. grandis varies in its susceptibility to colonization by different Pisolithus isolates in a manner that is not predictable by geographic origin or the ITS-based phylogeny of the fungal partner. Further, elevated levels of CO₂ alter the receptivity of E. grandis to Pisolithus, which is correlated to a dramatic shift in the transcriptomic profile of the root. These data provide a starting point for understanding how future environmental change may alter the signalling between plants and their ectomycorrhizal partners and is a step towards determining the mechanism behind observed shifts in eucalypt-associated fungal communities exposed to elevated levels of atmospheric CO₂.

Regimes of temperature variability for microbial adaptation to ocean change
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Photosynthetic marine microbes are the foundation of ocean ecosystems. By virtue of their microscopic size, microbes experience the ocean as a viscous medium, and their motion is therefore determined by drift with the ocean currents. Temperature has a direct influence on microbial photosynthesis and metabolism and when the ambient temperature exceeds optima for individual species, this can result in sharp declines in growth. Up until now, the analytical framework for understanding the thermal exposure of microbes has typically involved a Eulerian view – evaluation of temperature fluctuations at fixed points in space. However, for ocean microbes, this framework does not take into account their transport in dynamic seascapes, implying that our current view of change for these critical biota may be inaccurate. In this presentation I will show that upper ocean microbes experience-long-trajectory temperature variability up to 10°C greater than the seasonal variability estimated in a static frame, and that this variability depends strongly on location. These findings demonstrate that advection has the capacity to influence marine microbial community assemblies, such that taxa with narrow temperature performance curves will potentially be restricted to regions of the global ocean where ocean currents are weak or along-trajectory temperature variation is low. The results shift our present understanding of microbial operational temperatures and have implications for understanding the costs and constraints of adaptation.

Unravelling microbial bioremediation of organophosphates: a metagenomic systems-biology approach
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The growing extent of contaminated land and water due to industrialization is a major global concern and the remediation of contaminated sites is a key goal of a sustainable future. Organophosphate (OP) compounds represent a major environmental contaminant due to their role as a nerve agent and thus widespread application as pesticides. In addition to acute toxicity to humans, which results in over a million poisonings annually, chronic exposure to these compounds may lead to long term neurological and developmental effects. Microbial bioremediation of toxic compounds often suffers from a lack of understanding...
of the types and roles of degrading bacteria within the context of the diverse community and ecological drivers present in the system. New technologies are leading to a more holistic understanding which synthesizes information regarding the organisms present, functional gene abundances and environmental variables. Here we have used shotgun metagenomics to profile microbial communities from agricultural soils across a gradient of OP insecticide usage and varying in their ability to degrade OP compounds in situ. We found that the major determinant of both taxonomic composition and genomic functional potential was a site history of insecticide usage. These shifts were driven by an increase in genes related to phosphorus metabolism, membrane transport, stress response and chemotaxis. These metabolisms were also found to increase in abundance after the addition of OP insecticide to laboratory samples which previously had low concentrations of these compounds. By applying network analysis to this dataset, we have built a model that links key taxa to OP degrading genes within a wider metabolic network to elucidate the consequences of organophosphate pesticide on microbial community structure and function, and to predict strategies to encourage the degradation of these compounds in situ leading to bioremediation of contaminated sites.

Microscopic interactions with macroscopic effects: Do microorganisms create plant diversity in rainforests?

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Rainforests harbor remarkable plant species richness and understanding the source of rainforest diversity is a long-standing question in plant-community ecology. The Janzen-Connell hypothesis proposes that rainforest diversity could be maintained by the selective inhibition of seedlings that germinate near conspecific neighbors. Because common species have a higher probability of gerninating near conspecifics, this mechanism stops common species from coming to dominate a community, whilst simultaneously providing interspecific species with an advantage and preventing their local extinction. Recent observations of experimentally manipulated plant communities have implicated microorganisms, specifically fungi, as agents that selectively inhibit the recruitment of common species. However, the microbial communities themselves remain largely unexplored. We report the first investigation into microbial communities associated with rainforest tree species and identify patterns in bacterial, fungal and oomycete community structure. The microbial community data was collated with extensive (spanning fifty years) plant community data collected from this study site to establish whether microbial communities have a role in the maintenance of rainforest plant diversity.

We demonstrate preliminary evidence that both bacterial and fungal factions of the community may have a role to play in the maintenance of rainforest tree diversity. We also demonstrate that major abiotic drivers of community structure act differently upon fungal and bacterial factions. Microorganisms likely to be responsible for the selective inhibition of common species leading to the maintenance of rainforest diversity are currently being identified through next gen sequencing.

Understanding how rainforests can stably support high levels species diversity, and the effect of changing environmental factors upon these communities, is central to our ability to conserve these ecosystems. The question of how diversity is maintained may also contribute to our ability to approach conservation at the level of ecosystems and help abate the flood of extinctions that are occurring at every trophic level.

Understanding the Parastagonospora nodorum - wheat interaction: is it as simple as we think?

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It has long been thought that necrotrophic pathogenic fungi use a barrage of lytic enzymes to break down plant cells to access the nutrients held within. In recent years it has emerged that some necrotrophic fungi possess a more complicated and specific infection strategy, appearing reliant on a gene-for-gene mechanism as observed in biotrophic pathogens. For the wheat pathogen Parastagonospora nodorum, it has been demonstrated that the basis of this host specific interaction is small cysteine-rich effector proteins secreted during infection (ToxA, Tox1 and Tox3). It is hypothesised that these effectors interact with specific dominant susceptibility genes in the host leading to a programmed cell death response and disease. However, whilst we now understand the requirement of these effector proteins for disease, their modes of action remain poorly understood. In this seminar, I will describe the mechanisms of these effector proteins and discuss the role they play in causing disease.

Macrophage-pathogen interactions: a metabolic perspective

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Paradoxically, several important human pathogens survive in the very cells equipped to kill them - the macrophages. Macrophages may thus provide unique environments that support intracellular survival and replication of pathogens. Pathogen growth is intimately linked to the ability to scavenge essential nutrients from the host. We provide evidence that this has led to the tropism of the protozoan parasite Leishmania to the highly lytic lysosomes of macrophages. Leishmania require sugars for survival in macrophages, however glucose levels within macrophage lysosomes are insufficient to support intracellular replication. We now show that Leishmania have access to amino sugars in macrophages and that intracellular parasites can utilize amino sugars as major carbon source. Macrophages readily take up and recycle extracellular cellular matrix polymers that are enriched in amino sugars. Leishmania are unable to utilize these polymers directly, but rather depend critically on host enzymes to generate free amino sugars. Given that this enables Leishmania growth in macrophages and to cause disease in animals, we propose a novel strategy to target intracellular pathogens.
Intravital imaging of leukocyte homing during Cerebral Malaria

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Cerebral malaria is a severe complication that occurs during Plasmodium falciparum infections in humans. Mouse models using Plasmodium berghei ANKA have been important for elucidating the precise molecular and cellular events involved in the progression of this disease. A key feature of cerebral malaria pathogenesis is the sequestration of leucocytes within the blood vessels of the brain, which is thought to contribute to vascular damage and occlusion and disease progression. Nevertheless, the precise spatial-temporal context of leucocyte behaviour in the central nervous system in vivo is poorly understood. Using intravital microscopy, we showed that both Ly6C+ monocytes and T cells are recruited to the pial vessels before the appearance of neurological signs. Within the vascular lumen, monocytes were occasionally found in close contact with regions of blood vessel associated with perivascular macrophages. Development of neurological signs was associated with increased retention of both Ly6C+ monocytes and T cells in the vascular lumen. At late stage disease, a small fraction of T cells was found to enter the brain parenchyma where they actively migrate. In contrast, monocytes were restricted to the vascular lumen. Our studies map the sequence of leucocyte accumulation in the vasculature during cerebral malaria, and highlight hitherto unknown cellular interactions between monocytes, T cells and perivascular macrophages.

Fungal inositol pyrophosphate IP₇ is crucial for host-pathogen interaction and virulence

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Inositol molecules phosphorylated with mono- and diposphates (IPs and PP-IPs respectively) perform multiple key functions in eukaryotes. However, their role in fungal pathogens has never been addressed. We investigate IP/IP- biosynthesis pathway in a model pathogenic fungus, Cryptococcus neoformans, which causes life-threatening meningoencephalitis. Phenotypic analysis of IP and PP-IP-deficient mutants combined with gene expression profiling identifies IP₇ (PP-IP₇) generated by the IP₇ kinase Kcs1 as a key signaling molecule in C. neoformans. Absence of this crucial metabolite affects cell wall integrity, melanization, mating and virulence in worm, mouse and murine models of cryptococcosis. IP₇-deficient (Δkcs1 mutant) cells fail to be recognized and internalized by monocytic THP1 cells and blood-derived monocytes, as compared to WT C. neoformans. At the gene expression level, the absence of IP₇ leads to elevated expression of protein biosynthesis machinery and reduced expression of genes encoding enzymes of citric acid and glyoxylate cycles, fatty acid β-oxidation pathway and gluconeogenesis. Furthermore, expression of multiple genes encoding transmembranal proteins, particularly sugar transporters, and secreted proteolytic enzymes is lower in Δkcs1, as compared to wild type. Taken together, our findings establish Kcs1-generated IP₇ as a major regulator of cellular metabolism affecting nutrient acquisition and surface properties of the fungal cell, and therefore crucially important for host-fungus interaction and virulence.

Morphological and metabolic adaptation to environmental conditions by Talaromyces marneffei and its role in the host.

Alex Andrianopoulos

Talaromyces marneffei (Penicillium marneffei) is an important fungal pathogen of humans, in particular those who are immunocompromised. T. marneffei has the capacity to alternate between a hyphal and a yeast growth form, a process known as dimorphic switching. The strongest extrinsic trigger for dimorphic switching is in response to temperature. T. marneffei grows in the hyphal form at 25°C and in the yeast form at 37°C. The hyphal form produces conidia, which are likely to be the infectious agent, and believed to establish infection after inhalation. The yeast growth form is the pathogenic form found in infected patients. These yeast cells exist intracellularly in the mononuclear phagocyte system of the host.

T. marneffei is the only true pathogen in a genus comprising a large number of species and is also the only dimorphic fungus in this group. Yet there are a number of other fungi in more distantly related orders which also exhibit the capacity to alternate between hyphal and yeast growth forms. Many of these are also pathogens of animal or plants. As an intracellular pathogen, T. marneffei must be able to utilise the available nutrient sources in order to grow while evading or tolerating the host’s defence systems. The results from a number of lines of investigation into the molecular control of the dimorphic switch and the events which establish and maintain the morphological states in T. marneffei, both of which are central to understanding pathogenicity, will be presented.
Endophytic pathogens, water stress and dieback in an invasive tree

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Dieback is prevalent in many populations of invasive woody weeds globally. There are many preventative and contributing biotic and abiotic factors related to dieback occurrence1. Previous dieback studies have focused on specific potential causative biotic agents, but the majority remain unexplained. Parkinsonia aculeata L. (parkinsonia), an invasive tree in northern Australia. It has naturalised across a wide range of climatic zones in northern Australia2. Many of which experience long periods of extensive drought interrupted by extreme rain events. Parkinsonia dieback has been observed in both drought-affected and regions prone to flooding3 but little is known about whether or not water availability has a role to play in dieback occurrence.

In a glasshouse trial we tested the interactive effects of water availability and fungal inoculation on growth and pathogenesis of parkinsonia. We sampled roots, stems and stem tips from healthy and dieback-affected parkinsonia from northern Queensland. Fungal isolates from the samples were cultured and identified via ITS sequencing. A number of which experience long periods of extensive drought interrupted by extreme rain events. Parkinsonia dieback has been observed in both drought-affected and regions prone to flooding3 but little is known about whether or not water availability has a role to play in dieback occurrence.

We observed lesion formation and gummosis in parkinsonia inoculated with Pestalotiopsis mangiferae, P. clavipora, Lasiodiplodia pseudothespormae and Botryosphaeria dothidea and satisfied Koch’s Postulates, however we did not observe systemic infection, typical of dieback. Normal and inundated treatments were associated with larger lesion formation and increased gummosis. Lower water levels were also correlated to less plant growth, as indicated by plant height, dry weight and stem girth.

Parkinsonia dieback is a complex phenomenon, occasionally attributed to specific fungal pathogens. We have shown, however, that water availability and subsequent plant stress contributes to overall plant health and therefore susceptibility to infection by pathogens.


Molecular Epidemiology and Antigen Profiling of Neisseria meningitidis in Western Australia from 2000 to 2014

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Neisseria meningitidis is the causative agent of meningococcal disease which has a mortality rate of 6%. Multi-locus sequence typing (MLST) of seven housekeeping genes classes meningococci into genetic lineages termed clonal complexes (cc). A new vaccine, BEXSERO®, incorporates four main meningococcal surface antigens: factor H binding protein (fHbp), Neisserial Heparin Binding Antigen (NHBA), Neisserial adhesin A (NadA) and porin A (PorA). The vaccine is most effective against isolates expressing antigenic variants fHbp-1, PorA serosubtype 1.4, and NadA-1,2,3. Based upon antigenic variation and different expression levels of these proteins by isolates, most studies predict a vaccine coverage of 66-88% of disease causing isolates within each jurisdiction.

The aim of this study was to analyse the sequence variability of meningococcal antigens and predict the coverage by the BEXSERO® vaccine in Western Australia over the past 15 years.

The genomic DNA of 278 meningococcal strains isolated from patients was sequenced using Illumina paired ends. The short read sequence typing program (SRST2) was used to determine the allele of each MLST and antigen locus.

The majority of isolates (43%) belonged to cc41/44 has declined in prevalence as has the overall disease. In the entire collection, 51%, 30% and 15% respectively possessed variants of fHbp-1, NadA-1,2,3 and PorA serosubtype 1.4. All strains encoded a gene for NHBA. Thus, 64% of isolated strains 2 or more of the vaccine antigens. fHbp-1 prevalence predominated all other types pre-2005, but underwent temporal shifts post-2005. In 2008 and 2012, fHbp-3 predominated whilst the prevalence of fHbp-2 remained steady across the sample period.

In conclusion, the predicted coverage of strains by BEXSERO® in Western Australia is similar to other reports worldwide. However, this study is the first to observe temporal shifts of fHbp over a 15 year time scale.

Microevolution of E. coli O157:H7 in a herd of Australian cattle

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Escherichia coli O157 can cause haemorrhagic colitis and haemolytic-uremic syndrome (HUS). They are often found as normal flora of ruminant animals such as sheep and cattle, allowing these organisms to be released into the
environment. Single nucleotide polymorphisms (SNPs) have been widely used to determine genetic relatedness and evolutionary relationships of E. coli O157 populations. However, SNPs are not very discriminatory as a previous study found that Australian O157 belonged to one SNP clade (clade 7). A more discriminating method, multilocus variable number tandem repeat (VNTR) analysis (MLVA) has been widely used and standardised for outbreak detection of O157. In this study, we analysed 169 E. coli O157 isolates from a herd of 23 cows obtained during a nine month period using both SNP typing and MLVA. SNP typing showed that 163/169 were clade 7, with six isolates belonging to a novel clade.

MLVA separated the 169 isolates into 32 unique MLVA profiles, with five predominant profiles. Isolates taken from the same cow did not always belong to the same profile, with all cows excreting isolates from at least two different MLVA profiles. The majority of profiles differed by either one repeat or one VNTR, with the most divergent profile differing by 5 VNTRs. The average number of alleles of the eight loci was 6 ranging from 2 to 19. By minimum spanning tree analysis three of the 5 dominant MLVA profiles were closely related with one repeat difference, likely to have evolved from one to another during the sampling period. Two MLVA profiles obtained during the late sampling period differed from the early sampled profiles by 2 or more alleles suggesting independent introductions. In conclusion, over a period of 7 months, the O157:H7 clone underwent microevolution and dynamic clone turnover.


DEVELOPMENT AND VALIDATION OF A SEROTYPE SPECIFIC QUANTITATIVE PCR ASSAY FOR DETECTION OF EQUINE ADENOVIRUS-2

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Diarrhoea is one of the most common conditions requiring veterinary intervention in foals and is a significant economic burden on horse industry in Australia. Along with several other infectious causes of equine diarrhoea, equine adenovirus 2 (EAdV-2) has been associated with gastrointestinal infections. Sensitive detection of equine adenovirus-2 is essential in the diagnosis of this virus and differentiation from adenovirus virus-1. In this study, a quantitative polymerase chain reaction (qPCR) assay was developed to amplify a 109 bp fragment of EAdV-2 hexon gene using SYBR Green for detection and specific identification EAdV-2 nucleic acid. The standard curves generated using serial dilutions of the cloned hexon gene demonstrated a broad dynamic range below 7 x 10^7 and 2.7 x 10^10 copies per reaction with correlation coefficient (R²) of 0.994 and 106.0% efficiency. The assay had a high reproducibility with low intra and inter-assay variations at all dilutions. The analytical specificity of the assay was confirmed using a range of equine viruses (equine adenovirus-1, equine herpesvirus-5, equine rhinitis virus B1) and no specific amplification was observed. The qPCR assay can detect 10 fold less starting DNA than conventional PCR. Overall, the qPCR developed is highly sensitive, reproducible and specific. This assay will be useful in ultimately enhancing our understanding of EAdV-2 infection as well as simplifying the detection of this virus.

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Biofilms – hidden environmental reservoir of antibiotic resistant microorganisms in ICU

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Health Pathology

Hospital acquired infections (HAIs) are a burden on healthcare systems and patients in terms of lost bed days and additional treatment costs for such infections. Multifaceted infection control protocols are in place to minimise the acquisition of such infections however, the contamination of hospital environmental surfaces has been underestimated as an impacting factor. Previous studies have demonstrated the presence of multi drug resistant biofilms on hospital surfaces. Microbial growth in biofilm structures provides protection against disinfection and also provides a reservoir for microbial release of cells which could result in infection if contact with a contaminated surface occurs. High touch surfaces were identified using adenosine triphosphate (ATP) bioluminescence readings. The presence of ATP is an indicator of a soiled surface. Surfaces with high ATP bioluminescence readings were destructively sampled from the Intensive Care Unit (ICU) of Liverpool Hospital, NSW and examined for the presence of multi drug resistant microorganisms (MDROs). MDROs such as vancomycin resistant enterococci (VRE) and extended spectrum beta lactamase (ESBL) bacteria were grown from these samples and identified using MALDI-TOF and VITEK for antibiotic sensitivities. VRE environmental isolates were compared to clinical strains collected from ICU patients using ERIC PCR and were found to have similar/identical banding patterns. Samples of surfaces with confirmed MDROs were imaged using scanning electron microscopy and were found to have biofilm growth. These findings indicate that these multi resistant biofilms are showing resistance to current cleaning protocols and prove difficult to remove via conventional cleaning methods. As the VRE isolates had similar/identical banding patterns to the clinical strains from the ICU, this indicates these biofilms may provide a reservoir of multi resistant bacteria, potentially leading to HAI development. A biofilm targeted cleaning protocol may aid in the removal of these environmental contaminants and may lead to a reduction in HAI acquisition.
Sequencing of PCR positive Giardia and Campylobacter reveals novel genotypes

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We have validated Giardia PCR positives using 9 different gene targets. Although each assay designed was capable of detecting virtually all the sequences for that target in the public databases, the number of Giardia samples detected using most of the assays was quite low suggesting a high degree of variability in the sequence of Giardia genes. The variability of Giardia species found in human samples was supported by mapping the Giardia assemblage. When using GDH as a PCR target some additional positives were observed that had a sequence that was most closely related to Giardia, but did not correspond to any sequence found in the databases. This suggests that a species closely related to Giardia exists that has not yet been sequenced. A similar situation occurred when validating Campylobacter positives using primers that were designed to detect all Campylobacter species. A wide diversity of Campylobacter species were detected in Australian clinical setting including some that were not present in any public database, but still most closely related to Campylobacter. We conclude that with the power of modern molecular diagnostics the complexity of infectious pathogens will increase.

Leaky lytic gene expression is an integral part of herpes simplex virus latency

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Herpes simplex viruses (HSV) are highly successful human pathogens that have long provided the paradigm for latency/reactivation as a strategy for survival in a host population. It is now clear that HSV lytic gene transcription persists at a low level into latency, but whether this leads to protein production has not been shown. We examined HSV activity during the establishment of HSV latency in mice (between 5 and 30 days after infection) and then later using a mouse model in which LacZ expression is switched on permanently in cells experiencing HSV-driven Cre recombinase expression. A panel of viruses with Cre under the control of various promoters in HSV enabled counting of infected sensory neurons that had experienced different types of HSV activity; Entry of a virus genome was examined by driving Cre from the CMV IE promoter and we chose four lytic gene promoters (ICP0, ICP47, RR1 and gB) to track expression of these HSV genes. Surprisingly, the appearance of marked neurons and spread of virus to sensory ganglia not innervating the infected dermalome continued to increase for 5-6 days beyond the time when infectious virus load peaked. In addition the promoters for ICP47, RR1 and gB (an immediate early, an early and a late gene, respectively), but not ICP0 (a second immediate early gene) were active and able to drive Cre protein expression during latency, ICP47 was especially interesting, being highly active during the establishment of latency. These data suggest that i) the traditional kinetic classes of HSV genes are a poor guide to HSV gene expression in neurons in vivo and ii) residual lytic gene expression leads to protein production, providing antigen to drive ongoing adaptive immune responses. We conclude that lytic gene expression needs to be understood as a part of any unified model of HSV latency.

Engineering E.coli for growth on organochlorine pollutants

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Organochlorine compounds are widely used in industry, and are persistent pollutants. 1,2-dichloroethane (DCA) in particular is a problem locally as a groundwater contaminant at the Botany Industrial Park in south Sydney. Although some naturally-occurring bacteria can biodegrade DCA, such isolates are difficult to genetically manipulate, and are not easily amenable to strain improvement (e.g. to expand their substrate range). Thus, we aimed to construct a synthetic DCA biodegradation pathway in E.coli, to allow us to better understand the evolutionary processes that lead to bacterial growth on xenobiotics, and to enable directed evolution approaches to be used to enhance the activity of the DCA-degrading bacteria.

We used three DCA biodegradation genes from Xanthobacter (dhaA, dhaB, aldA) and one human metabolic gene (adh1b1) to construct our pathway. The NAD-requiring human alcohol dehydrogenase gene was used here since the corresponding Xanthobacter gene requires an unusual cofactor not found in E.coli (PQQ). The four catabolic genes were cloned into a single plasmid under the control of the tetracycline promoter.

Inducible co-expression of active AldA, DhlB and DhlA were all successfully achieved in response to tetracycline induction, as revealed by enzyme assays and SDS-PAGE. Tetracycline-induced cells effectively degraded both DCA and chloroacetate (a key pathway intermediate). However, no expression of Adh1B1 protein was observed, and the recombinant E.coli could not grow on DCA. Sequencing revealed a point mutation in the adh1b1 gene introduced during cloning, which may have caused the metabolic pathway to be incomplete. This study provides an excellent foundation for the construction of a complete heterologous DCA pathway, with 3 out of the 4 genes cloned together and proven functional.

A systems approach for the identification of proteins from Mycoplasma hyopneumoniae required for biofilm formation

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Mycoplasma hyopneumoniae (Mhp) is a chronic respiratory pathogen that causes significant losses to swine production worldwide. Disease involves colonisation of the respiratory tract cilia, causing ciliostasis and epithelial cell death. An understanding of the molecular mechanisms that underpin Mhp virulence is needed to develop efficacious vaccines. We show for the first time that Mhp can form biofilms on abiotic and biotic surfaces. This alternate lifestyle involves
Ecology of *Listeria* species on Australian farm environments suggests a key role for aquatic niches in contamination routes

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Identifying contamination patterns on dairy farms is critical to controlling the potential transfer of undesirable microorganisms into both the animal herd and raw milk. In the case of *Listeria* species, this is particularly important as L. monocytogenes can cause severe illness in both humans and the dairy herd; *L. ivanovii* causing infection in the dairy herd. This study monitored contamination routes of *Listeria* species across 7 dairy farms located throughout Victoria. This included identifying contamination patterns across farms, and the state, through sampling at locations such as water supplies, farmland pastures, animal feed, the dairy herds, as well as raw milk. Pulsed-Field Gel Electrophoresis (PFGE) was utilised to examine the molecular ecology of the *Listeria* population. A diverse population of *Listeria* species was identified, with *L. innocua* (n=19) isolated most frequently, followed by *L. seeligeri* (n=6) and *L. ivanovii* (n=4), while *L. monocytogenes* (n=1) and *L. grayi* (n=1) were least common. Population analysis identified multiple clones present in geographically diverse locations throughout the state. Aquatic niches were a significant feature of the isolate distribution across farms, with frequent contamination of environmental water reservoirs utilised by farms as a main water source. Isolate contamination could be tracked from these water reservoirs through to feeders for animals. Mobile genetic elements conferring resistance to heavy metals were identified, suggesting adaptation to heavy metals in the environment may be a feature of the population; 16% of isolates showed high resistance to cadmium. Results suggest groundwater may be an important distribution chain for *Listeria* species throughout the state. This study provides evidence for harbourage sites and contamination routes of *Listeria* species in farm environments across Victoria, and identifies the emerging water supply as a key entry route for *Listeria* into the farm environment. This knowledge will help direct future control strategies to help minimise the potential spread of *Listeria* into the farm system, and ultimately into raw milk and the dairy food chain.

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**Gluatthione and DNase I disrupt *Pseudomonas aeruginosa* biofilms in a medium mimicking cystic fibrosis sputum and regulate expression of *P. aeruginosa* virulence genes**

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*Pseudomonas aeruginosa* is the predominant bacterial species infecting the sputum and lungs of adults with cystic fibrosis (CF) and is difficult to eradicate with traditional antibiotic treatment. One of the hallmarks of *P. aeruginosa* colonisation is its ability to form strong biofilm matrix that inhibit antibiotic penetration. *P. aeruginosa* biofilm matrix primarily consists of exopolysaccharides, extracellular DNA (eDNA), and the virulence factor pyocyanin. *P. aeruginosa* infected CF lung secretions (sputum/mucus) contain a significant amount of pyocyanin (up to 27 µg/ml) and eDNA (3-14 mg/ml), compared to none in uninfected lung secretions. The recent finding that the *P. aeruginosa* metabolite pyocyanin intercalates with eDNA to strengthen the biofilm has led to a search for inhibitors of pyocyanin binding and eDNA accumulation. The antioxidant glutathione ([GSH] directly binds to pyocyanin, thereby disrupting its’ association with eDNA, whilst DNase I non-specifically degrades DNA. We investigated the transcriptomic effects of GSH and DNase I on acute and chronic isogens of the Australian Epidemic strain-1 in artificial sputum medium (ASMDM) to imitate CF lung conditions. Total RNA was extracted from 48hr biofilms at 8hr post-inoculation with 2 mM GSH and 50 units DNase I, and depleted for rRNA. mRNA transcripts (n=3) were tiled and sequenced using RNA-sequencing and compared to those of a non-GSH control of the strain grown simultaneously under the same conditions. Results showed disruption of the biofilm with GSH resulted in upregulation of genes associated with acute infectivity, including pyocyanin biosynthesis, indicating that *P. aeruginosa* is able to adapt to removal of its biofilm by reversion from sessile to planktonic lifestyle. This is an important finding in the evaluation of combined inhaled glutathione with DNase I for treatment of chronic *P. aeruginosa* infections in individuals with CF.

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Identification of transcription factor HLH3 as the master regulator of phosphate acquisition and storage in the human fungal pathogen, *Cryptococcus neoformans*

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*P. aeruginosa* is the predominant bacterial species infecting the sputum and lungs of adults with cystic fibrosis (CF) and is difficult to eradicate with traditional antibiotic treatment. One of the hallmarks of *P. aeruginosa* colonisation is its ability to form strong biofilm matrix that inhibit antibiotic penetration. *P. aeruginosa* biofilm matrix primarily consists of exopolysaccharides, extracellular DNA (eDNA), and the virulence factor pyocyanin. *P. aeruginosa* infected CF lung secretions (sputum/mucus) contain a significant amount of pyocyanin (up to 27 µg/ml) and eDNA (3-14 mg/ml), compared to none in uninfected lung secretions. The recent finding that the *P. aeruginosa* metabolite pyocyanin intercalates with eDNA to strengthen the biofilm has led to a search for inhibitors of pyocyanin binding and eDNA accumulation. The antioxidant glutathione ([GSH] directly binds to pyocyanin, thereby disrupting its’ association with eDNA, whilst DNase I non-specifically degrades DNA. We investigated the transcriptomic effects of GSH and DNase I on acute and chronic isogens of the Australian Epidemic strain-1 in artificial sputum medium (ASMDM) to imitate CF lung conditions. Total RNA was extracted from 48hr biofilms at 8hr post-inoculation with 2 mM GSH and 50 units DNase I, and depleted for rRNA. mRNA transcripts (n=3) were tiled and sequenced using RNA-sequencing and compared to those of a non-GSH control of the strain grown simultaneously under the same conditions. Results showed disruption of the biofilm with GSH resulted in upregulation of genes associated with acute infectivity, including pyocyanin biosynthesis, indicating that *P. aeruginosa* is able to adapt to removal of its biofilm by reversion from sessile to planktonic lifestyle. This is an important finding in the evaluation of combined inhaled glutathione with DNase I for treatment of chronic *P. aeruginosa* infections in individuals with CF.

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Identification of transcription factor HLH3 as the master regulator of phosphate acquisition and storage in the human fungal pathogen, *Cryptococcus neoformans*
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**Background and aims** Genes encoding plasma membrane transporters of free phosphate (Pi) are essential for virulence of the deadly meningococcal meningoencephalitis-causing fungal pathogen Cryptococcus neoformans and are up-regulated during infection, suggesting that the pathogen encounters and responds to Pi-limiting conditions in vivo. However, the transcription factors responsible for regulating expression of genes encoding these transporters and proteins involved in mobilizing Pi from extra- and intracellular sources, are unknown.

**Methods and Results** Here we used a colorimetric enzyme assay which measures the hydrolysis of para-nitrophenyl phosphate (pNPP) to screen a C. neoformans transcription factor (TF) knockout library for mutants that fail to secrete Pi-repressible acid phosphatase activity derived from Aph1 (the Saccharomyces cerevisiae Pho5 homologue). The screen identified the putative TF, HLH3, which contains the basic helix-loop-helix (HLH) domain present in 3CPho5. Using qPCR we demonstrate that all phosphate transporters (PHO4, PHO5, PHO9) and Aph1 fail to be induced in the HLH3 mutant (hnh3Δ), consistent with the inability of hnh3Δ to mobilize and import Pi from the environment. Growth, and expression of 3 genes encoding intracellular acid phosphatases and 3 genes involved in Pi release from phospholipids, were also abrogated in hnh3Δ during Pi starvation, consistent with Pi mobilization from intracellular sources is also compromised in hnh3Δ.

**Conclusions** In summary, we have identified HLH3 as the master regulator of Pi homeostasis in C. neoformans and shown that it controls the expression of known and novel Pi-responsive genes involved in mobilization of Pi from external and intracellular sources.

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**What will climate change mean for infectious disease? The soil perspective**

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Scientists have long predicted large-scale responses of infectious diseases to climate change, giving rise to a polarizing debate, especially concerning human pathogens for which soil-ecological drivers and control measures can limit the detection of climate-mediated changes. Climate change has already increased the occurrence of diseases in some natural and agricultural ecosystems, but in many cases, outcomes depend on the form of climate change and details of the host-pathogen system. Here, we describe how climate change will affect terrestrial ecosystems and their capacity to reduce infectious diseases. Rhizosphere and bulk soil was collected from grassland, forest and agricultural ecosystems at the Hawkesbury and EUC-FACE climate change field and greenhouse experiments in Western Sydney (Australia). Real-time PCR approaches targeting toxins-encoding genes revealed that elevated CO₂ and rainfall patterns intensified the effect of warming by significantly increasing the virulence of soil-borne human pathogens associated with grassland and forest rhizosphere and bulk soils. As opposed to simply increasing the biomass of soil-borne pathogens at ambient CO₂ under changes in rainfall patterns and temperature, elevated atmospheric CO₂ strongly selected for virulent human pathogens and affected shifts in pathogens composition. 16S rRNA, 18S rRNA and ITS region sequencing with the Illumina Miseq platform revealed the dominance of several opportunistic and true human pathogens in the rhizosphere microbiome, including E.coli 0157:H7, Enterobacteriacae, Chlamydia, Staphylococcus, Salmonella and Clostridium species. The potential mechanisms involved in the interplay between human pathogens in the rhizosphere microbiome are presented in a bioclimatic model of relative microbial abundance that specifically incorporates interactions between biological units.

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**Role of the inositol polyphosphate kinase Ipk1 in the pathogenesis of Cryptococcus neoformans**

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Cryptococcus neoformans (Cn) is the leading cause of fungal meningitis worldwide. Cn utilizes a number of signalling pathways to aid its survival within a human host and regulate the expression of key virulence-related traits. We have identified a novel signalling pathway in Cn, involving the phosphorylation of inositol triphosphate (IP3) to more complex inositol polyphosphates (IPs) and inositol pyrophosphates (PP-IPs) by a series of inositol polyphosphate kinases (IPKs).

To determine the role of Ipk1 (a putative IP3 kinase) in IP homeostasis and its contribution to the virulence profile of Cn, an Ipk1 gene deletion mutant (CnΔipk1) was created using PCR and biolistic transformation. HPLC revealed that IP3 accumulating in CnΔipk1, consistent with Ipk1 functioning as an IP3 kinase.

Phenotype characterisation of Δipk1 showed an attenuated virulence phenotype: a cell wall defect, reduced laccase/urease activity, and reduced secretion of the phosphate (Pi)-repressible acid phosphatase (Aph1) activity in phosphate limiting conditions due to reduced induction of Aph1 gene expression. Δipk1 was hypersusceptible to antifungals including amphotericin B and theazole drug family. Δipk1 was hypovirulent in a mouse inhalation model of cryptococcosis over a 64 day infection period but established a persistent low-grade asymptomatic lung infection. Δipk1 was not readily phagocytosed by THP-1 cells and failed to activate them to the same extent as the wild-type strain as assessed by flow cytometry and qPCR respectively. For qPCR, an RT-profiler array designed for analysis of the antifungal immune response was used. In conclusion, CnΔipk1 is an IP3 kinase required for the expression of virulence-related traits in vitro, the promotion of drug tolerance and pathogenicity in vivo. These results suggest that IP species produced down-stream of Ipk1 are involved in regulating virulence and phosphate homeostasis, and studies into these mechanisms are ongoing.
Drivers’ Disconnect: Deterministic processes vary during community assembly for ecologically dissimilar taxa

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The continuum hypothesis states that both deterministic and stochastic processes contribute to the assembly of ecological communities. However, the contextual dependency of these processes remains an open question that imposes strong limitations on predictions of community responses to environmental change. We measured community and habitat turnover across multiple vertical soil horizons at 183 sites across Scotland for two microbial groups, both dominant and functionally vital components of all soils, that differ substantially in their growth habit and dispersal capability (bacteria and fungi). We found that habitat turnover was the primary driver of bacterial community turnover in general, although its importance decreased with increasing isolation and disturbance. Fungal communities, however, exhibited a highly stochastic assembly process, both neutral and non-neutral in nature, largely independent of disturbance. These findings suggest that increased focus on limitations to dispersal and biotic interactions within assemblages are necessary to manage and conserve the key ecosystem services provided by these assemblages.

Copper (II) Lead (II) and Zinc (II) inhibit the growth, reproduction and rate of attachment to organic substrates of four zoosporic fungi species from soils of NSW

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Zoosporic true fungi (chytrids) are widely distributed in soils. They reproduce by motile spores (zoospores), which attach to, and grow saprophytically on many substrates of plant and animal origin, such as pollen, keratin and chitin (Sparrow 1960). Here we investigate the in vitro effects of soluble Copper (II), Lead (II) and Zinc (II) on the zoosporic true fungi species: Rhizophyctis rosea (A13), Tarenymyces sp. (A3) and Chytromyces hyalinus (A14) from soils of the Sydney Basin and Central coast regions and Gaertneriomyces (Mar-CC2) from a soil of north-western NSW. The growth, zoospor production and attachment of all isolates showed toxicity to soluble metals in the following order; Cu>Zn>Pb. All isolates showed significant reduction in growth at 60 ppm (0.94 mmol m⁻³) for Cu, three declined significantly at 60 ppm (0.92 mmol m⁻³) Zn and two declined significantly at 100 ppm (0.48 mmol m⁻³) Pb. All isolates showed reduced zoospore production when grown in solid PYG media with 60 ppm Cu, three isolates declined in zoospore production at 60 ppm Zn and three at 100 ppm Pb. Two isolates did not recover growth after incubation in 60 ppm Cu, while all isolates recovered growth after incubation in solid media with 60 ppm Zn or 100 ppm Pb. If these metals cause similar effects in the field, Cu, Pb and Zn contamination of NSW soils is likely to reduce the biomass of zoosporic true fungi and reduce attachment to organic materials, thereby reducing the rate of mineralisation of soil organic matter.

Challenges and opportunities: whole genome sequencing in public health microbiology

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Next generation sequencing technologies has brought whole genome sequencing (WGS) into reach of many clinical and public health microbiology laboratories. These areas face a number of specific challenges as they implement the use of high throughput genomics in not only outbreak investigations but also for routine molecular surveillance.

The Queensland reference laboratory, Public Health Microbiology (PHM), is uniquely positioned for bacterial outbreak investigations containing specialist public health water, food and clinical microbiology sections. The Molecular Epidemiology team provides molecular support to all areas and is the key contributor to bacterial molecular diagnostics, epidemiological surveillance and outbreak responses.

The integration of WGS into PHM over the last three years has been successful in improving efficiency and capabilities. WGS has been utilised for the real time investigation of number of bacterial outbreaks including a nosocomial Legionella cluster and Salmonella and S. aureus water and food outbreaks. WGS analysis assessed against conventional molecular typing for routine epidemiological surveillance of bacterial diseases such as N. meningitidis and L. monocytogenes has proven to be successful and sustainable. There are many advantages offered by this approach, however the challenges around implementing NGS technology in a public health laboratory are ongoing and will benefit from national coordination.

One Health and Superbugs: the ever growing threat from foods and water

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Antibiotic resistance is rapidly rising internationally. Many bacterial infections are now very difficult, and sometimes impossible, to treat. Antibiotic resistance is proportional to use. The more antibiotics used, the more resistance develops and spreads.

With many common bacteria infecting people, transmission is almost entirely human-to-human (e.g. pneumococcus). Thus any antibiotic resistance seen in these bacteria, will be almost entirely due to antibiotics used in the human sector. However with some other bacteria, the resistance seen is almost entirely due to what has occurred in the agriculture sector in food animals. Examples are non-typhoid Salmonella and Campylobacter, which in developed countries are almost always acquired via foods. Vegetarians are also at risk because water, food crops and fields (via manure) can

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be contaminated with resistant bacteria. There are other bacteria where the risk of acquisition from animal sources is more difficult to precisely define, but appears to be quite important. Examples are E. coli and Enterococcus.

Antibiotics have been used for over 60 years as growth promoters in animals. They are also used in large amounts for prophylaxis (called “metaphylaxis”) when entire herds are treated at the same time. Huge numbers of animals are treated continuously for most of their life with either in-feed or in-water antibiotics. Some of these “prophylactic” antibiotics are “critically important” classes for humans, such as the fluoroquinolones and 3rd and 4th generation cephalosporins (WHO).

While there is ongoing debate on the exact quantitation of the amount of antibiotic resistant bacteria infecting people coming from food animals, it appears to be large. In Australia fluoroquinolone use is banned in food animals and we have one of the lowest fluoroquinolone resistance rates in campylobacter, salmonella and E.coli.

There is overwhelming evidence around the world that using antibiotics on crops, in aquaculture and in food animals, causes resistant bacteria to develop in large numbers and that these bacteria are transmitted to people via foods and water. When these resistant bacteria infect people they cause increased deaths and morbidity. Decreasing the total amounts of antimicrobial used in people and agriculture, stopping antibiotics being used as growth promoters, stopping the use of “critically important” antibiotics in food animals, decreasing corruption in countries and improving water quality around the world are all part of a “One Health” approach that if followed will make major contributions to controlling antimicrobial resistance.

Anncaliia algerae microsporidal myositis in Australia

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The microsporidian, Anncaliia algerae is an environmental organism that primarily infects insects. Although the mechanism of transmission leading human infection is unknown, this may occur when there is contact with water that contains spores. Anncaliia algerae was first reported in 2004 as a cause of fatal myositis in an immunosuppressed person with rheumatoid arthritis from the USA. Since then, 4 other cases have been described. All of the subsequent patients were from coastal New South Wales, and were treated with immunosuppressive agents. Two had a history of rheumatoid arthritis and two had solid organ transplantations. Symptoms and signs were indicative of a skeletal muscle myositis. However, peripheral nerves and other organs may have been involved. Microsporidia of the Anncaliia genus were identified in skeletal muscle biopsies using electron microscopy. Organism DNA was amplified with polymerase chain reaction, and sequence analysis confirmed the diagnosis of A. algerae. The patient who survived had a reduction in immunosuppressive therapy, measures to prevent complications and a regimen based on albendazole. Anncaliia algerae has emerged as a cause of myositis in Australia and there is ongoing investigation of the local epidemiology.

Molecular Strategies for Gonococcal Antimicrobial Resistance Surveillance in Australia

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In 2013 the United States Centres for Disease Control Threat Report rated Gonococcal AMR as an urgent Public Health Threat. The treatment guidelines from the United States, The United Kingdom and Europe recommend dual treatment with ceftriaxone and azithromycin for uncomplicated gonorrhoea. This is also the recommendation for the majority of Australia; however, in some remote regions of Australia, amongst the indigenous population resistance rates are very low, and gonorrhoea acquired locally or in an endemic region can be treated with oral penicillin based protocol. In these remote areas antimicrobial resistance (AMR) testing is difficult however molecular tests have been developed and implemented to enhance isolate based surveillance.

In 2013, in Australia, decreased susceptibility to ceftriaxone was reported in 8.6% of isolates, double that reported in 2012 (4.4%). The highest proportions were reported from New South Wales and Victoria where the greatest increases in disease notifications occurred. Genotypic data generated by members of the NNN show that strains harbouring a mosaic penicillin-binding protein 2 – considered a pivotal mechanism for cephalosporin resistance – now comprise approximately 8% of all gonococcal infections in Australia. Further, in 2013, a new multidrug resistant gonococcal strain (A8806) with a ceftriaxone MIC of 0.5mg/L, the highest ever reported in Australia, had key genetic similarities to a rare ceftriaxone-resistant strain, observed in only a single case in Asia. Enhanced surveillance for the A8806 strain was implemented.

In summary, gonococcal disease rates and AMR rates are increasing, and in 2013 the proportion of strains with elevated ceftriaxone MIC values has doubled since 2012. For the first time in Australia, ceftriaxone resistance and high level resistance to azithromycin has been reported. The next direction for treatment is uncertain, but what is clear is that gonococcal AMR poses a serious public health threat and disease prevention and disease control programs are urgently called for, and that continued monitoring of AMR to inform treatment and monitor interventions is paramount. Molecular surveillance is now in place to enhance isolate based surveillance in Australia.
Operational performance of a new molecular-based point-of-care test for diagnosis of Chlamydia trachomatis and Neisseria gonorrhoeae infection: concordance with conventional laboratory testing

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Background
New molecular-based point-of-care (POC) tests for Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (NG) infections are being used for the first time by trained Aboriginal health workers/practitioners, registered/enrolled nurses and medical officers in regional and remote health services in Australia as part of the TTANGO (Test, Treat And GO) trial. We assessed the operational performance of the GeneXpert® CT/NG assay (Cepheid) POC test at these services using conventional laboratory tests as the reference standard.

Methods
TTANGO, a randomised cross-over control trial of CT/NG POC testing, commenced June 2013. To date, 12 services have implemented GeneXpert testing on-site as routine practice, with specimens continuing to be sent to jurisdictional laboratories for conventional nucleic acid amplification testing (NAAT) as usual. We assessed the concordance of GeneXpert performed by health service staff with conventional laboratory NAAT. We also present selected details of discordant specimens.

Results
Among 1995 GeneXpert tests performed, CT and NG were detected in 182 and 127, respectively, by the jurisdictional laboratory. Concordance for CT was 99.4% (95% CI: 99.0 – 99.8) and NG was 99.9% (99.6-100.0). The fourteen discordant results (eight urines, six lower vaginal swabs) were identified in seven services and five laboratories [two use Cobas 4800, three use Aplima]. Discordant results were predominantly CT[n=12] and most[n=10] were positive POC/negative laboratory results. The median POC test crossing point among CT discordants was 37.2(IQR:31.6-37.7) with five of nine(55.6%) having crossing points>35, compared to 29.2(IQR:26.3-32.6) among CT concordants with 10 of 17(5.5%) having crossing points>35. The two NG discordant results were both positive POC/negative laboratory results.

Conclusion
The operational performance of GeneXpert CT/NG in the hands of trained health service staff is excellent and consistent with previous laboratory and field evaluations. Higher crossing points of discordant results most likely indicates low organism loads close to test detection threshold and seem unrelated to service, laboratory, specimen type or reference assay. Overall, results show the GeneXpert method is suitable for routine detection of CT and NG.

Salmonella Typhimurium shedding and egg contamination in experimentally infected hens

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Salmonella is a major cause of foodborne illness worldwide. Most cases of human salmonellosis are associated with the consumption of contaminated eggs and egg-related products. Globally Salmonella Enteritidis is the most predominant serovar responsible for egg associated Salmonella outbreaks. In Australia Salmonella Typhimurium (S. Typhimurium) is a major cause of egg associated outbreaks although, mechanism of egg contamination is not yet established. In this study, laying hens were orally challenged with S. Typhimurium at week 14 with either S. Typhimurium phage type 9 (PT9) (T group) or S. Typhimurium PT9 and S. Mbandaka in combination (MT group). Faecal samples of infected hens were processed for Salmonella isolation and enumeration by most probable number (MPN) method at day 1, 3, 6, 9, 12, week 3, 5, 7, 9, 11, 13 and 15 post infection. Eggs laid during week 5, 7, 9, 11, 13 and 15 were tested for the presence of Salmonella on egg shell surface and egg internal contents. Salmonella was recovered from faeces of infected hens on all days but the overall mean Salmonella counts were significantly higher in MT group (32.47±3.12) compared to T group (21.58± 3.20). The overall frequency of egg shell contamination was also higher (18.43%) in MT group as compared to T group (5.66%). None of the internal egg contents were positive for Salmonella throughout the study. The results of this study suggest that S. Typhimurium PT9 was able persist in gut of infected laying hens until week 15 post infection. The shedding of S. Typhimurium on egg shells was variable across 15 weeks of post infection.

Identifying the true terIS end of ISCR1?

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ISCR1, first identified as a “common region” (CR) in some class 1 integrons, and other related ISCR elements have been responsible for capturing and mobilising certain antibiotic resistance genes. They are unusual insertion sequences, related to the IS911 family. Members of this family lack the terminal inverted repeats found in many other IS families and they move by rolling circle replication, catalysed by the Rcr protein encoded within the element. Replication starts at the 5’ end, downstream of the rcr gene, and continues through the element to terIS motifs defining the other end. Insertions of IS911 family elements in different locations allowed their ends and target sites to be defined, as well as identifying cases where repication has continued beyond the terIS end, resulting in capture of adjacent DNA segments. ISCR1 has always been found adjacent to the same position of the 3-conserved segment of class 1 integrons, making it hard to define the exact terIS end. It has been suggested...
that this structure could be explained by a deletion encompassing part of an ancestral ISCR element and part of the 3'-CS. However, at 2,154 bp the element defined as ISCR1 appears longer than other ISCR elements (e.g. ISCR2 is ~67% identical to ISCR1 over 1,339 bp) and could already contain captured segment(s) adjacent to the original terS. Searches with the Roc1 protein identified an ISCR1-like element in a plasmid from an uncultured bacterium, also recently identified in association with the acc(6')-lan amikacin resistance gene on a plasmid from Serratia marcescens and found with this gene in a plasmid from Escherichia coli and two Acinetobacter baumannii genomes. An alignment of the nucleotide sequence of this element with ISCR1 revealed 79% identify over 1,811 bp and a pair of short inverted repeats, a characteristic of terS of IS91-like elements, is present near to the end of this match. Identification of an element related to the region currently defined as ISCR1 appears to support the hypothesis that this region may contain an ancestral ISCR plus additional segment(s) adjacent to terS.

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Replication of staphylococcal multiresistance plasmids

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Theta-replicating, low copy number plasmids have contributed significantly to the evolution of antibiotic resistance in Staphylococcus aureus. These plasmids act as an assembly point for the rise of large antimicrobial resistance gene clusters, which are then capable of spreading to other bacteria as a single entity by horizontal DNA transfer mechanisms.

Most S. aureus multiresistance plasmids have been found to carry an evolutionarily related replication region that encodes a replication initiation protein (Rep) belonging to the RepA_N family. The Rep protein from conjugative multiresistance plasmid pSK41 initiates plasmid replication by binding to four directly repeated sequences in the origin of replication (oriV) found at the centre of the rep gene. The DNA-binding domain is located in the N-terminal 120 residues of Rep. The role of the Rep C-terminal region has yet to be determined; however, phylogenetic analyses indicated a higher level of sequence conservation in plasmids from the same genera suggesting that the C-terminal region could interact with host-encoded protein(s). The Rep-oriV nucleoprotein complex presumably causes strand melting at oriV, followed by the assembly of host-encoded proteins to form the replisome, although the intricacies of these molecular processes in pSK41 replication are unknown.

In this study, we utilised the yeast two-hybrid system to investigate potential protein-protein interactions that occur between the Rep protein and host-encoded replication proteins. An S. aureus genomic DNA library was constructed and screened using pSK41 Rep as the bait. We detected a direct interaction between pSK41 Rep and DnaG primase, a primosomal protein essential for DNA synthesis. As a component of the primosome, DnaG continually synthesize the primers for Okazaki fragments during lagging-strand replication. In pSK41 replication, DnaG may also play a role in priming of the leading strand. DnaG is also known to interact directly with DnaB, and thus, could assist in the recruitment of other replisome components to the pSK41 oriV.

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Functional analysis of the conjugation system of Clostridium perfringens

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Functional genetic analysis of the novel tcp locus has revealed that it mediates the conjugative transfer of the toxin and antibiotic resistance plasmids from Clostridium perfringens. This locus has nine conserved genes that are required for efficient conjugative transfer of the paradigm tetracycline resistance plasmid, pCW3. We have now examined the role of the unique membrane proteins encoded by the tcpD and tcpE genes and have shown that they are essential for conjugation. Localisation studies using functional HA-tagged TcpD and TcpE derivatives in C. perfringens showed that TcpD and TcpE localised to the cell envelope, mostly at the poles of donor cells, suggesting that they form part of the transfer apparatus. Surprisingly, neither a relaxase nor an origin of transfer (oriT) has been identified on pCW3. The first gene of the tcp locus, intP, encodes a putative lysosome recombinase. An intP mutant was shown to have a significantly lower conjugation frequency and complementation with the wild-type intP gene in trans restored transfer to wild-type levels. An intP-Y259F derivative was unable to restore transfer, demonstrating that the Y259 residue was essential for intP function, which was consistent with the need for a hydrophilic attack at the oriT site. In addition, a putative accessory protein, TcpK, was identified as a potential component of the pCW3 relaxosome and shown to be required for wild-type pCW3 transfer. Finally, mobilisation studies defined the pCW3 oriT site as a 148 nt region downstream of the intP gene. Gel shift assays showed that intP bound specifically to the oriT site and that a supershift was observed in the presence of both IntP and TcpK. In conclusion, we postulate that IntP is an atypical relaxase that together with TcpK initiates conjugative transfer by binding to and processing the oriT site.

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Exposure to sub-clinical concentrations of antibiotics induces genomic and phenotypic changes and promotes antibiotic resistance

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Antibiotics and the genetic elements that confer resistance to them are simultaneously released into aquatic environments via human waste streams and agricultural runoff. These antibiotics persist at low, but biologically relevant, concentrations that could induce a general increase in the frequency of point mutations, recombination, and lateral gene transfer via the SOS response. Here we examined the response of Pseudomonas aeruginosa and Pseudomonas protegens to sub-clinical levels of the antibiotics kanamycin,
tetracycline and ciprofloxacin. Single bacterial colonies were serially streaked onto media containing 1/10 the minimum inhibitory concentration (MIC) of each antibiotic. Serial transfers were then maintained over 40 generations. To monitor for possible cross-contamination of cultures, we performed ERIC and BOX PCR every five generations. To our surprise, significant alterations to the PCR fingerprints were detectable after only five transfers, suggesting indels, transpositions, or point mutations had altered the PCR targets in the experimental lines. No such changes occurred in control lines. Experimental lines also displayed variant colony morphologies by generation 40. Final MICs were significantly higher in some experimental lines of Ps. protegens, suggesting that 1/10 the MIC was sufficient to select for antibiotic resistance. The implications of these results are clear: Exposure of the environmental microbiome to low level antibiotic pollution will induce genomic changes, including the potential to generate newly resistant species that may be of significant concern for human health.

### Horizontal gene transfer of Chlamydial tRNA modification enzymes to parasitic protozoa: An untapped resource of putative drug targets?

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Antimicrobial drugs used in bacterial infections exploit the differences in cell biology between prokaryotic and eukaryotic cells to target the bacterial pathogen, while its host remains largely unaffected. Infections caused by eukaryotic pathogens, such as parasitic protozoa, are often more difficult to treat due to the highly similar cell biology between the pathogen and its eukaryotic host. This complication could be overcome by searching the genomes of parasitic protozoa for genes that are: 1) important for cell growth or virulence 2) absent from the genome of their host and 3) of bacterial origin. We have identified two gene families encoding tRNA modification enzymes in several eukaryotic microbes, which were acquired from the Chlamydiae family of obligate intracellular bacteria via horizontal gene transfer. These proteins localise to various compartments of the cell including the mitochondrion, chloroplast and endoplasmic reticulum. Among the eukaryotic microbes that possess these bacterial proteins are the oyster pathogen Perkinsus marinus as well as the clinically-relevant human pathogens Entamoeba histolytica and Acanthamoeba castellanii. The chemical modifications catalysed by tRNA modification enzymes are important for tRNA function, translation efficiency and in some cases virulence. The bacterial origin and horizontal acquisition of these enzymes also means they are absent in their respective hosts. Taken together, these horizontally acquired enzymes have potential for the development of drugs that inhibit their activity with no effect on the host. We hypothesise that the development of inhibitors that specifically target these enzymes may lead to novel strategies to treat or slow the progression of E. histolytica and A. castellanii infections as well as provide significant economic savings for oyster farms that are plagued by P. marinus. Thus, prokaryote-to-eukaryote horizontal gene transfer represents an untapped resource of potential drug targets in pathogenic eukaryotes.

### The dynamics of chromosome components that promote genome stability: Centromeres, Telomeres and Origins of replication

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Genome dynamics provide a rapid mechanism of stress response, yet ultimately chromosomes must be relatively stable. The non-coding chromosome components: centromeres, telomeres and origins of replication are all important for this genome stability. Centromeres are instrumental in assuring proper chromosome segregation and are likely to play a key role in the missegregation events that lead to aneuploidy as well as...
High gene and genomic variation revealed in the re-sequencing of 13 isolates of the fungal wheat pathogen Zymoseptoria tritici
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Zymoseptoria tritici is a necrotrophic pathogen of wheat, with a long asymptomatic period, followed by extensive plant necrosis and active pathogen growth. The mechanisms through which the fungus rapidly induces host cell death remain largely unknown and is currently hypothesized to be mediated by the secretion of small proteins or metabolites. These molecules are commonly referred to in fungal pathogens as effectors. This work seeks to exploit variation at the genomic level on a differential set of 13 isolates known to vary in virulence towards a range of Australian wheat cultivars in order to identify effector gene candidates. We use whole genome re-sequencing to identify single nucleotide polymorphisms and probable presence/absence polymorphisms in coding regions throughout the genome. This analysis has identified over 1 million SNPs and over 3,000 putative gene absences in at least one of the 13 genomes. The rate of gene absence is significantly higher on the eight accessory chromosomes when compared to the 13 core chromosomes. We have developed a python tool, PhyB, which rapidly sorts through gene trees in order to match genotypic variation with known virulence phenotypes.

Discovering the Protein Interaction Code: post-translational modifications that modulate protein-protein interactions
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Methylation of proteins occurs predominantly on arginine and lysine residues in the eukaryotic cell. Until recently, its predominance was unknown and its role obscure. This presentation outlines our efforts to construct the first ‘methylproteome network’ for a eukaryotic cell and presents evidence that methylation modulates protein-protein interactions in this network.

We analysed the yeast methylproteome to identify methylated proteins and precise modification sites. Targeted data acquisition - electron transfer dissociation LC-MS/MS was used, as were yeast proteome arrays (containing 4,400 chips spotted on to microscope slides). To build the intracellular methylation network, all known and putative methyltransferases in yeast were knocked out and the methylproteome re-analysed to determine which enzyme was responsible for which methylation event. Enzyme-substrate links were first investigated by the analysis of recombinant substrate proteins methylated by recombinant enzymes, by in vivo methylation assays and/or the incubation of proteome arrays with recombinant enzymes. Validated enzyme-substrate links were integrated with yeast-protein-protein interactions to generate the first ‘methylproteome network’. A new “conditional two-hybrid” (C2H) system was then constructed to test whether methylation can modulate protein-protein interactions.

Our analyses, together, showed that protein methylation is widespread in the eukaryotic cell. We discovered two new eukaryotic lysine methyltransferases, elongation factor methyltransferases 2 and 3, both of which have mammalian orthologs. Our integrated methyltransferase-substrate protein and protein-protein interaction network suggested that methylation can modulate protein-protein interactions. This was proven by our new “conditional two hybrid” system, in that half of the protein-protein pairs involving arginine methylated proteins show a significant increase in interaction on methylation. In some cases, phosphorylation was found adjacent to methylation and we have shown that this can interfere with methylation and thus decrease certain protein-protein interactions. To conclude, the ‘methylproteome network’, built here, reveals a novel means of regulating protein-protein interactions and thus biological function in the eukaryotic cell.

Teaching an old target new tricks: Focusing on purine biosynthesis in antifungal development
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Purine nucleotide biosynthesis and homeostasis plays a critical role in most cellular processes, including signal transduction, protein translation, DNA replication, RNA synthesis, and energy metabolism. While this extremely well conserved and characterized pathway plays a crucial role as a therapeutic target in humans, most famously for immunosuppressants and anticancer agents, it has not been well studied as a potential target for the development of antifungal drugs. Exploiting this, we have utilised a combination of genetic, biochemical and structural biology approaches to investigate the viability of purine biosynthesis as a chemothapeutic target for the treatment of life-threatening disseminated fungal infections. Beginning with the rate-limiting enzyme required for de novo GTP synthesis, IMP dehydrogenase (IMPDH), and the key salvage enzyme required for the scavenging of guanine, hypoxanthine-xanthine-guanine phosphoribosyltransferase, we initiated a comprehensive dissection of the entire purine biosynthetic pathway in the fungal pathogen Cryptococcus neoformans. We found that de novo GTP biosynthesis, but not salvage, is limiting under certain conditions and could be a potential therapeutic target in vivo. In contrast to other organisms, the IMPDH mutant displays a variety of phenotypic defects including
slow growth and attenuated virulence factor production. With the aid of X-ray crystallography and detailed steady-state enzyme kinetics we initiated a large-scale drug screen, identifying lead compounds with the potential to serve as the basis of fungal-specific IMDH inhibitors. As the IMDH story continues, we have expanded our study of the de novo purine biosynthetic pathway, methodically creating deletion mutants, determining crystal structures and developing detailed kinetic profiles of additional enzymes in the pathway. Together, these results are not only supporting the potential of purine biosynthesis as a viable antifungal target, but also helping us develop a deeper understanding of this important primary metabolic pathway in the fungi.

The Koala (Phascolarctos cinereus) Faecal Bacterial Microbiome; Does Change in Diet Impact Community Structure?

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The koala (Phascolarctos cinereus) is an obligate dietary specialist, due to its ability to overcome a difficult diet that is nutritionally challenging and toxic to many animals. Their diet is composed almost exclusively of Eucalyptus, which has evolved a wide range of plant secondary metabolites, many of which function as effective defenses against herbivory. Relatively little is known about the composition and diversity of the gut microbiome of koalas, despite their importance in extracting nutrients from a challenging diet. Thus, knowledge of microbes driving the gut microbiota of koalas ingesting specific diets would assist in development of targeted inoculations, given prior to translocation or, release after rehabilitation.

This study investigated the microbiome of 33 wild koalas from Cape Otway, Victoria, Australia, using DNA extracted from faecal samples collected from two time-points. We utilised the Illumina MiSeq platform together with the QIIME pipeline and PRIMER v6 to identify significant differences in the gut microbiota within one population of animals known to separate into two diet types. Analysis of cuticular n-alkanes indicated the presence of a T6SS with high homology to S. algae grown under a diverse range of growth conditions. Genomic analysis of S. algae identified the presence of a T6SS with high homology to that of O1/O139 serotype Vibrio cholerae. To investigate the activity of this T6SS, the SA1 proteome was harvested to characterize the type VI secretory system of Gram-negative bacteria.

The development of a proteogenomic pipeline to characterise the type VI secretory system (T6SS) of Gram-negative bacteria

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The type VI secretion system (T6SS) is a recently described bacterial export pathway that has been characterised in a range of Gram-negative organisms. The pathway is involved in the export of a variety of effectors that can target both prokaryotic and eukaryotic cells. While bioinformatic analysis of sequenced genomes shows that T6SS elements appear widespread in nature with 25% of Gram-negative bacteria harboring 1 or more copies, functional analysis has only been carried out for a handful of organisms. The functional data that has been published shows that the T6SS exhibits an impressive sophistication and complexity. Importantly, the T6SS has been shown to be involved in bacterial pathogenesis of clinically important pathogens, such as Escherichia coli, Vibrio cholerae and Pseudomonas aeruginosa.

In order to understand the biological significance and role played by T6SS elements in cell-to-cell interactions, we have carried out a proteogenomic characterisation of the T6SS from Shewanella algae (S. algae) grown under a diverse range of growth conditions. Genomic analysis of S. algae identified the presence of a T6SS with high homology to that of O1/O139 serotype Vibrio cholerae. To investigate the activity of this T6SS, the SA1 proteome was harvested following growth on a range of complex media (LB broth, LB agar, blood agar, blood heart infusion broth and TCBS agar) and investigated utilizing 1D-SDS-PAGE and LC-MS/MS. The results demonstrate that the expression of T6SS proteins in S. algae are differentially expressed and can be activated via growth on solid media containing components derived from eukaryotic cells. This systems biology approach allows us to present a comprehensive multi-genome analysis of genetic loci harbouring gene clusters constituting the key differentially regulated T6SS elements.
POSTER ABSTRACTS

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**Novatec Novalisa™ versus DiaSorin Liaison® XL Assays for the serological detection and diagnosis of Borrelia burgdorferi.**

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Borrelia burgdorferi sensu lato is a spirochete bacterium that causes Lyme disease in humans. Lyme disease is a tick-borne illness endemic in North America, Europe and Asia. Lyme disease manifests clinically with mild flu-like illness, skin lesions, neurological involvement and arthritis. Many patients are asymptomatic however. Early clinical presentations of Lyme disease may display erythema migrans (EM) lesion, a localised homogenous expanding rash that spreads into larger circular ‘bulls-eye’ appearance. Multiple lesions may be present in early disseminated stages. If untreated, patients may develop monarticular and oligoarticular arthritis for several years later.

Serological evidence of Lyme disease may be absent in early stages as IgG and IgM antibodies to Borrelia burgdorferi may take up to 8 weeks to develop.

Pathology North, Royal North Shore Hospital recently completed a second evaluation on the kits from DiaSorin and as a result of the second round of evaluations, the laboratory is now comfortable to perform Borrelia burgdorferi serology the DiaSorin Liaison XL.

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**Allele-specific multiplex-microsphere qPCR genotyping of Campylobacter jejuni**

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Worldwide, Campylobacter bacteria are associated with food-borne diseases. So far, while more than twenty species of Campylobacter have been recognised, Campylobacter jejuni remains the most relevant pathogen. The main infection source is raw chicken, but cases including cattle and domestic animals, such as cats, have been reported recently [2]. Infection with Campylobacter can cause diarrhoea and can be critical for patients of higher risk, such as elderly people, and immunocompromised patients. In these critical cases, the gold standard treatment is the administration of antibiotics. However, antibiotic susceptibility is challenged by the emergence of antibiotic resistance [3]. Tracking the source of an infection as well as understanding the spread of the organism in animal production systems are important steps in reducing the impact of this organism and require effective, efficient typing methods.

The current gold standard method for typing is multilocus sequence typing (MLST). MLST, however, is not applicable to the standard laboratory due to the lack of cost efficiency and time consumption [4]. Therefore, we performed Campylobacter jejuni genotyping by allele-specific multiplex-microsphere quantitative PCR detection within 4 hours [1]. A high resolution of genotypes is possible as the single-nucleotide polymorphisms (SNP) within the MLST genes targeted are selected on the basis of highest possible Simpson’s index of diversity (D). This new approach is currently being extended to include presence/absence assays (e.g. antibiotic resistance and virulence genes) to improve the power of the typing. In other applications, we are exploring direct detection and typing of pathogens in other complex diseases, such as sepsis.


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**Same Day MALDI-TOF Identification of Bacteria from Positive Blood Culture Bottles: Evaluation of Two Methods**

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**Introduction**

The advent of MALDI-TOF mass spectrometers has increased the pressure in clinical microbiology laboratories to identify bacteria faster than ever before. In this study, two methods that enabled the same day identification of bacteria from positive blood culture bottles (Bact/Alert FAN Plus, bioMérieux) by MALDI-TOF (Bruker) were evaluated in parallel with the standard overnight culture method.

**Method**

Using a ‘short-incubation’ method, 2 drops of blood culture broth was inoculated onto a chocolate agar plate, without streaking, and incubated for 4-hours (35°C CO₂). MALDI-TOF testing was performed from the inoculum. Alternatively, the ‘pellet’ method used a Vacuette gel tube inoculated with 5ml of blood culture broth. The tube was centrifuged at 2000 rpm for 15-min, the supernatant discarded and the pellet used to inoculate the MALDI target plate using a cotton swab.

Only organisms commonly isolated in this laboratory with identification to the species level (MALDI-TOF score values of ≥2) were included in this evaluation. *Streptococcus pneumoniae* and mixed Gram-stain results were also excluded from the data.

**Results**

During the period 30/09/2014 – 09/04/2015, 376 bottles were included in the evaluation. The short incubation and the pellet method respectively identified 89.4% & 64.6% of *Enterobacteriaceae*, 42.9% & 0% *Pseudomonas aeruginosa*, 81.0% & 19.0% *S.aureus*, 17.9% & 9.0% of coagulase-negative
Evaluation of BioMerieux VITEK MS MALDI-TOF and Bruker biotype for identification of microorganisms isolated from clinical specimens

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Introduction
Rapid identification of microorganisms in clinical laboratory is important for patients' management. Bacterial identification by conventional methods takes about 48 hours after the receipt of specimens. However, matrix assisted laser desorption/ionization-time of flight (MALDI-TOF) is a relatively newly developed technique for rapid identification of microorganisms with high accuracy and low operating costs. We evaluated two different MALDI-TOF equipments with different platforms for identification of microorganisms in clinical specimens.

Materials and methods
For evaluation of accuracy, 143 and 193 clinical specimens were tested with Vitek MS and Bruker biotype respectively and the results were compared with the ones obtained by Vitek II. And for evaluation of precision, 16 and 20 American Type Culture Collection (ATCC) standard microorganisms were repeatedly tested daily for 20 days with Vitek MS and Bruker biotype respectively.

Results
The concordance rate of Vitek MS was 91% with 130 concordant results and 13 discordant. Among the discordant results, gram negative was highest with 13% (11/84), one in gram positive (5%, 1/21) and yeast (3%, 1/38) respectively. Precision was 98% with 315 good identification, 1 low discrimination and 4 no identification. The concordance rate was 91% in Bruker biotype with 176 concordant and 17 discordant results. Among discordant results, yeast was highest with 20% (2/10), gram negative 11% (11/97) and gram positive 5% (4/85). The precision was 98% with 432 good identification, 8 probable genus identification and 1 not reliable identification.

Conclusions
MALDI-TOF is accurate and precise tool for identification of microorganisms with reduced turnaround time and operating cost compared to conventional methods.
A comparison of the efficiency of commercially available automated nucleic acid extraction platforms to detect a wide range of respiratory viruses from a bank of clinical samples

Ineka Gow, Shoo Peng Siah, Angela Elmore, Dylan Warby, Damien J Stark, Jock Harkness, John R Melki, Douglas S Millar

Objective: Molecular diagnostic techniques (MDx) are becoming increasingly prevalent in molecular microbiology laboratories and are especially useful for the detection of viral pathogens, which have traditionally been diagnosed by enzyme immunosorbent assays (EIA) or tissue culture. MDx are particularly useful due to the rapid turn around time and increased specificity achieved compared to conventional technologies. Automation of viral nucleic acids extraction for downstream detection techniques, usually PCR based, markedly reduces hands-on time for laboratory workers and improves workflow. We sought to compare a wide range of commercially available systems in their ability to detect a bank of respiratory samples.

Methods: Nucleic acids were extracted from 100 primary clinical samples using a range of commercially available platforms including the Qiasymphony (Qiagen), Kingfisher Flex (Thermo), EasyMag (Biomerieux), MagNApure 96 (Roche) and Nimbus (Hamilton). Samples were extracted according to the manufacturers recommendations and PCR carried out using the Genetic Signatures EasyScreen™ Viral respiratory detection kit. Amplification reactions were carried out on a Biorad (Hercules) CFX384 machine. Each batch of samples from each system were ran simultaneously on the same PCR plate to ensure no variation, thus amplification efficiency could be determined precisely for each extraction instrument.

Results: Clinical samples were ran on each platform in order to give a representation of samples that would be received by routine testing laboratories. Platforms differed markedly in terms of both positivity and inhibition rates. The full details of the results obtained from each system will be discussed.

Conclusion: A wide range of nucleic acid extraction platforms are available and differ in sample throughput, instrument and reagent costs, footprint and whether the platform is open or closed. The data demonstrated that variations were observed with each platform in terms of the sensitivity for the detection of commonly encountered respiratory viruses. Users should be aware that results will differ between extraction systems and that extraction of nucleic acids is just as important as downstream PCR performance.

Comparison study of The Alere BinaxNOW Legionella Urinary Antigen Card with Quidel Sofia Legionella FIA and Trinity Biotech Uni-Gold Legionella Urinary Antigen PLUS for use in a Microbiology Laboratory setting

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Three Urinary Antigen Test kits the Alere BinaxNOW Legionella Urinary Antigen card, Quidel Sofia Legionella FIA and Trinity Biotech Uni-Gold Legionella Urinary Antigen PLUS were compared for sensitivity, specificity, ease of result interpretation and general ease of use. A total of 30 urine samples were tested with all three methods. Further a positive titration endpoint was determined to be the same with all three assays. The Quidel Sofia Legionella FIA and Alere BinaxNOW legionella Urinary Antigen card showed comparable result sensitivity (100%) and specificity (100%), the Trinity Biotech Uni-Gold Legionella Urinary Antigen PLUS showed a lower sensitivity (85%) and comparable specificity (100%). The Trinity Biotech Uni-Gold Legionella Urinary Antigen PLUS required more staff handling and subjective reading of the antigen band, the Binax assay was generally easier to use but also required subjective reading of the antigen band. The Sofia instrument provided an automated result not requiring manual interpretation but is limited to single unit analysis and a time management strategy for multiple testing. All three assays are acceptable for use for detecting L. Pneumophila serogroup 1 from urine.

Mycoplasma pneumoniae: A Three Year Quality Assurance Review

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Mycoplasma pneumoniae is a human pathogen that causes a form of atypical bacterial pneumonia, and is involved in upper and lower respiratory illnesses. It is a bacterium in the shape of a short rod, lacking a cell wall, which belongs to the class Mollicutes. The diagnosis of Mycoplasma pneumoniae is generally undertaken with serological testing or PCR, as the organism is difficult to grow in the laboratory. RCPAQAQ Serology offers quality assurance testing for Mycoplasma pneumoniae serology and the survey data from 2013 to 2015 has been reviewed here.

In the RCPAQAQ Serology Mycoplasma pneumoniae surveys in 2013, 2014 and 2015 between 79 and 83 participants returned results. Of these, approximately half are international participants. Results returned included IgG, IgM, IgA and Total Antibody testing by a variety of different methods including Enzyme Immunoassay (EIA), Chemiluminescence (ChLIA), Immunofluorescence (IFA), Particle Agglutination Test (PAT) and Complement Fixation Test (CFT). The majority of participants have performed IgG and IgM testing or Total Antibody testing only. From the first survey in 2013 to the most recent survey in 2015, we have seen a subtle shift in the testing of IgG and IgM with a move from the manual EIA methods towards the automated ChLIAM methods.

Of the 30 IgG, IgM or Total Antibody results that were reviewed in the five surveys, 24/30 (80%) obtained consensus (80% or greater agreement of the result). Of these, seven (23%) obtained 100% consensus of participant results. Discrepancies in the Total Antibody testing appear to be due to variable cut-off values (due to the subjective nature of the test), whereas the discrepancies in the IgG and IgM testing are due to the specimen type (plasma and heat-inactivated serum) or due to the result value being close to the cut-off. Overall participants have shown good concordance for the Mycoplasma serology surveys.
The microbial community of Eutherian origin
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Recent research challenges the accepted dogma, "that the fetus develops in a sterile environment" as molecular sequencing techniques, reveal the presence of microbial DNA in placental tissue and amniotic fluid collected from term and preterm deliveries. Furthermore, research investigating metabolic diseases has revealed a dysbiosis in the gastrointestinal microbial communities of obese individuals compared to lean individuals. It is unknown whether the presence of maternal obesity during pregnancy impacts on the maternal microbiota transferred to offspring.
Objective: To characterize the placental microbiome in term placentae collected from obese women compared to the placentae of healthy weight women.
Methods: Microbial DNA was extracted and purified as previously described. Illumina next-generation sequencing was used to characterize the placental microbiome from each cohort targeting variable regions in the 16s rDNA gene. Results: Preliminary data indicates a specific microbial community profile in placentae collected from obese women compared to healthy controls. Both healthy weight women and obese women demonstrated a microbial community dominated by Gammaproteobacteria. However, the relative abundance of members of the Firmicutes and Betaproteobacteria phyla demonstrated an inverse correlation between the two groups. Placentae collected from obese pregnant women harboured increased Betaproteobacteria and decreased Firmicutes when compared to healthy weight women.
Conclusions: Further analysis is required to investigate the potential significance of this finding in the context of the relationship between maternal and neonatal obesity.

High-throughput sequencing of chronic wounds: healing vs non-healing wounds
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Chronic wounds are a significant public health burden and associated with complex polymicrobial communities. Bacterial colonization has been hypothesized to be one of the main underlying causes of chronic wounds and leads to detrimental effects on wound healing. However, some members of the wound microbiota may also have beneficial effects on wound healing. The wound microbiome has been underappreciated because of the limitations of standard microbiological culture techniques. The aim of this study was to characterise the bacterial population in healing versus non-healing wounds and to identify possible biomarkers to predict wound healing trajectory by using culture independent next-generation sequencing. Wound swabs were sampled from 18 healing wounds and 30 non-healing wounds. High-throughput pyrosequencing with barcoded primers targeting the 16s rDNA gene was used to generate organism specific sequences. Sequence analysis and data mining was performed using QIIME and Calypso software programs. Principle Component Analysis revealed that there is a distinct difference in the microbial population associated with the healing vs non-healing wounds. Furthermore, OTUs (Operational Taxonomical Units) and taxonomic assignments identified 185 genera in both wound categories. Abundances of some bacterial genera/species were significant (p<0.05) in healing wounds and visa versa. These species can be considered as biomarkers for healing and non-healing wounds. In addition, this study suggests that the high abundance of species in non-healing wounds can be used to improve chronic wound management.

Quorum protection, cell survival and death
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For the growth of a cell culture, one inoculates not with one cell but with a quorum of cells. This is most often a requirement, not just a convenience, and most of us take this for granted without question. Here this observation is re-examined to understand why a quorum may be required to grow cells. The importance of quorums may be widespread in the aspects of microbiology they affect. It is very likely that quorums are connected with and have a large impact on the determination of Minimal Inhibitory Concentrations. It is also possible that low cell density may adversely affect cell survival, however, this is an area where even less is known. The need for a quorum might affect other aspects of microbial cell culture, cell isolation and cell preservation. Effects also extend to mammalian cell culture. Our recent studies explored the phenomenon of Candida glabrata cells requiring a quorum to survive. It was shown that cells release substances at less than 10 mg/L that I denote as quorum protection molecules. These substances are a complex mixture and are still to be fully characterized. Without them, cells at low density in water undergo death by apoptosis. More recent studies indicate that other yeast, including Saccharomyces cerevisiae utilise quorum protection. A better understanding of quorum protection and the molecules involved may enable more microbes from the environment to be cultured.

Cyclic-di-AMP levels in different Lactococcus strains is affected by CdaA and GdpP
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Chronic wounds are a significant public health burden and associated with complex polymicrobial communities. Bacterial colonization has been hypothesized to be one of the main underlying causes of chronic wounds and leads to detrimental effects on wound healing. However, some members of the wound microbiota may also have beneficial effects on wound healing. The wound microbiome has been
Cyclic-di-AMP is a ubiquitous bacterial second messenger playing a vital role in growth, cell wall homeostasis, antibiotic, heat- and salt-resistance. In Lactococcus, spontaneous mutation in the c-di-AMP phosphodiesterase gene (gdpP) caused a dual heat-resistant and salt-sensitive phenotype. Plating of a gdpP mutant on high salt agar resulted in the growth of a few colonies at low frequency. Genome sequencing of 2 selected salt resistant mutants revealed mutations in the gene encoding the diadenylate cyclase (CdaA) enzyme involved in c-di-AMP synthesis. Analysis of a further 100 salt resistant suppressor mutants led to the identification of >40 independent CdaA mutant variants. Those without mutations (n=4) were found to have revertant changes in the gdpP gene which likely restored phosphodiesterase activity. To confirm that these mutations affect c-di-AMP levels, we established an ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method to quantify cellular c-di-AMP levels. The results showed that intracellular c-di-AMP levels are undetectable in wild type L. lactis MG1363 and ~3-5x higher in a gdpP mutant compared to the cdaA/gdpP double mutants, demonstrating the c-di-AMP in Lactococcus is modulated by these enzymes. The inability to detect c-di-AMP in strain MG1363 could be due to this strain containing a natural frameshift mutation in the CdaA homolog encoding gene immediately downstream of cdaA. CdaR has been shown to strongly upregulate CdaA mediated c-di-AMP synthesis. We therefore analysed four cheese making strains and found that they all contain a complete unaltered cdaR gene homolog. However the level of c-di-AMP in one of these wild type strains (ASCC892185) was undetectable, while derivatives from it with gdpP and cdaA/gdpP mutations had high and low c-di-AMP levels, respectively. These results confirm that GdpP and CdaA control c-di-AMP levels in different strains of Lactococcus.
structure and function. If the sulfur cycling occurring within these communities can be disrupted, then the formation of sulfuric acid and consequently the corrosion of sewer infrastructure can be stopped. This is where the use of microelectromicrobiology can aid us in finding a potential solution to this widespread problem.

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**COMPARISON OF MICROBIAL COMMUNITIES ON PRE-PAINTED STEEL PANELS FROM AUSTRALIA AND MALAYSIA**

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Bacteria and other microorganisms have the ability to colonize most surfaces. Non-biological surfaces, such as painted surfaces encounter great challenges with respect to microbial infestation. Colonization of these microorganisms on painted surfaces causes aesthetic problems and may lead to the degradation of the materials and coatings. The aim of this study was to define the microbial communities that thrive in extremely challenging environments, particularly on the surfaces of roofs made from pre-painted steel strip. Fouling communities were compared across time as well as from different geographic locations in Australia and Malaysia. Pre-painted steel panels were deployed to the selected locations and exposed to the surrounding environment. The panels were collected every 4 weeks for 52 weeks. Panels were swabbed and total DNA was extracted using a modified CTAB-phenoI protocol. The 16S and 18S rRNA genes were amplified from DNA extracted from the panel surfaces for community analysis. Image based quantification of biofilm biomass on the surfaces of the panels showed an increase during the first quarter of the year before a decline of biomass after the first quarter. The overall diversity showed the dominance in occurrence of fungi (0.1- 4.7%) compared to bacteria (0.1-2.5%) for Australia samples. Samples from Malaysia showed infestation of fungi between 0.4-2.7% compared to bacteria 0.1-3.3%. Based on community analysis, the bacterial and fungal communities diversified with time. Observation of the study showed the fouling communities were influenced by time exposure instead of the geographic factors.

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Please stop wiping those instruments with alcohol

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In Brazil, some healthcare workers (HCW) believe that wiping surgical instruments with alcohol soaked swabs, post-use, will decrease biological soil and bacterial contamination of instruments, particularly if reprocessing is delayed. However, this practice is not recommended in disinfection and sterilization guidelines1-3. The aim of this study is to determine the effect of this practice on amount of soil and bacterial numbers on surgical instruments prior to processing.

**Methods**

Forty artery forceps were contaminated by soaking in tryplicity soya broth containing (TSB) 10CFU/ml of S. aureus or P. aeruginosa for 5 minutes. The forceps were allowed to air dry for 4 hours and subjected to various treatments with alcohol (80% v/v): none (control: 4 and 24 hours drying), wiping multiple instruments with one alcohol soaked swab, wiping a single instrument/soaked swab and spraying with alcohol. The instruments were left at room temperature for 20 hours. Bacterial numbers were determined following sonication in saline and standard plate culture. Residual soil was stained with crystal violet and quantified by spectrophotometry. The experiment was repeated twice.

**Results**

Bacterial contamination: Spraying instruments with alcohol decreased bacterial contamination (S. aureus 3 log reduction; P. aeruginosa 5 log reduction) compared to the controls. Reusing the same alcohol wipe for multiple forceps or changing the wipe between forceps had contamination to levels similar to the controls for S. aureus, but there was a slight reduction (1 to 2 log in both groups) for P. aeruginosa. Residual soil: neither wiping with alcohol soaked swabs nor spraying with alcohol decreased residual soil.

**Discussion**

Although spraying the instruments with alcohol decreased bacterial growth, probably due to better penetration of alcohol, residual soil remained high and similar to the control instruments. The practice of wiping instruments with the same alcohol soaked swabs for multiple instruments has two detrimental effects 1) possibility transfer bacteria from one instrument to another 2) alcohol acts as a fixative, increasing the difficulty in removing residual soil. Additional research and education is required in order to convince HCW to cease this practice.


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A Breath of Clean Air

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Tuberculosis (TB) is the second leading cause of death worldwide due to a single infectious agent. More than 95% of cases and deaths occur in low- and middle-income...
countries. In these resource-limited countries, hospitals are often lacking in adequate facilities for managing and isolating infected patients. Consequently there is a greater reliance on personal protective equipment, such as facemasks and respirators, to reduce nosocomial transmission of the disease. Facemasks are the most affordable and easily obtained option. However, healthcare workers in resource-limited settings are at an increased risk of becoming infected with TB due to the inability of hospitals to reliably provide new, sterile facemasks. Facemasks are sometimes reused despite their potential role in TB transmission, with decontamination not recommended due to concerns that sterilisation techniques may damage mask material.

Facemasks impregnated with an antimicrobial agent can add an extra level of protection against the spread of TB as they may decrease the risk of TB transmission and could be more cost effective if they did not need to be replaced as frequently. Conducting polymers (CP) and their functionalised derivatives (fCP) are a novel class of antimicrobial agents with potential as non-leaching additives to provide contamination resistant surfaces. We are investigating the antimicrobial action of a CP and an fCP against mycobacteria and have determined the optimal treatment time and concentration to achieve sterilisation of Mycobacterium smegmatis and Mycobacterium tuberculosis on a solid surface. Results indicated that an fCP is a potential candidate for use as an antimicrobial agent in facemasks to reduce transmission of TB.

### Predicted coverage and immuno-safety of a recombinant C-Repeat Region based Streptococcus pyogenes vaccine candidate

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The C-terminal region of the M-protein of Streptococcus pyogenes is a major target for vaccine development. The major feature is the C-repeat region, consisting of 35-42 amino acid repeat units that display high but not perfect identity. SV1 is a S. pyogenes vaccine candidate that incorporates five 14mer amino acid sequences (called J14 variants) from differing C-repeat units in a single recombinant construct. Here we show that the J14 variants chosen for inclusion in SV1 are the most common variants in a dataset of 176 unique M-proteins. Murine antibodies raised against SV1 were shown to bind to each of the J14 variants present in SV1. Additionally this sera reacted with five J14 variants not present in SV1. Antibodies raised to the individual J14 variants were also shown to bind to multiple but different combinations of J14 variants, supporting the underlying rationale for the design of SV1. Next, the immuno-safety of SV1 was assessed using a rat model of valvulitis. In this model, rat myocardial tissue displayed changes consistent with valvulitis following immunisation with control M5 protein, but not SV1. Together these results suggest that SV1 is promising vaccine candidate that will elicit antibodies that recognise the majority of M-types, and thereby may offer protection against most circulating strains.

### Degradation of Red Algae Substrate by Multi-enzyme Complexes Comprising of Agarase, Carrageenases and Neoagarobiolose Hydrodolase with Scaffolding Protein miniCbPA

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In the practice of converting red algal biomass into biofuel or valuable biochemicals, the critical step is the decomposition process of the agarose to give fermentable monosugars. In this study, we suggest enzymatic process that cost-efficiently produce biomaterials from red algae using multi-enzyme complex. These multi-enzyme complexes including three microbial chimeric enzymes (cAgaB, cCgkA and cAhgA) are stepwise convert the red algae biomass to fermentable sugars. We constructed chimeric gene containing the catalytic domain of β-agarase (AgAβ), neoagarobiolose hydrolase (AhgA) from Zoobella galactanivorans and κ-carrageenase (CgkA) from Pseudoalteromonas carrageenovora fused with dockerin domain from Clostridium cellulovorans by overlap PCR. The multi-enzyme complex including hydrolytic enzymes were formed with scaffolding protein miniCbPA via the cohesion and dockerin interaction. The multi-enzyme complexes were identified using non-denaturing PAGE analysis. And the analysis process of multi-enzyme complex was monitored by reducing sugar assay. As a results, by assembling, the protein band of multi-enzyme complex appeared as a single band molecular weight of 194 kDa. And we observed the increase of fermentable sugar production by multi-enzyme complex as 3.9-fold higher compared with the corresponding enzymes alone (cAgaB) in agar-carrageenan artificial substrates. Reducing sugar produced by the multi-enzyme complex was average at 17.7% higher produce than single enzymes average in all substrate combinations. Also, a combination of the three enzyme complex in substrate have synergistic effects, either enhancing or increasing the degree of polysaccharide breakdown and sugar release 33.5% compared to isolated other combination complexes. In the
light of these results, multi-enzyme complex will facilitate the biocconversion of useful products from red algae biomass which represents inexpensive and simple enzymatic process.

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**DMSP synthesis and cycling dynamics under increased light levels.**

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Accounting for 90% of ocean biomass, microorganisms form the foundation of the marine foodweb and are the engine-room for the chemical cycles that control our climate. However, human impacts, including pollution, modification and destruction of natural habitats and the influence of anthropogenically induced climate change have raised concerns about the future sustainability and welfare of marine ecosystems. Currently, we have limited knowledge of the effects of increased light irradiance on the phytoplankton population, bacterial community and chemical cycles. In particular the availability and uptake of the phytoplankton metabolite dimethylsulfoniopropionate (DMSP), which have been recently documented to drive many phytoplankton bacteria interactions. We have selected the coccolithophore Emiliania huxleyi to evaluate the effect of light and production of DMSP on the phytoplankton itself and on a natural bacterial assemblage.

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**Culture-independent genome sequencing and analysis of the chlamydial agent of epitheliocystis in Yellowtail Kingfish**

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**Background and Significance:** Novel families in the Chlamydiaceae have been associated with epitheliocysts, an under-recognised disease of fish. Phylogenetic analysis using 16S rRNA-based methods placed chlamydial pathogens of fish at the root of the order Chlamydiaceae, suggesting these organisms may be, or at least share similarity with, the evolutionary ancestors of the family Chlamydiaceae. Due to the lack of culture systems, little is known about the biology of these Chlamydia-like organisms (CLOs).

**Objectives:** In the absence of culture methods, we aimed to characterise the genome sequences of the novel pathogen, *Candidatus Parilichlamydia carangidicola* to better understand host-pathogen interactions, host adaptation mechanisms, and evolutionary relationships between CLOs and traditional Chlamydiaceae.

**Methods:** DNA was isolated from gills from two Yellowtail Kingfish presenting with chlamydial epitheliocysts. DNA was enriched for microbial DNA and whole genome amplification was conducted prior to shotgun sequencing on a MiSeq. The resulting reads were assembled using a de novo method (SPAdes). Genome annotation was conducted by RAST and genomes were analysed using a number of tools including Geneious, CLC genomics and DNAPlotter.

**Results:** We used a combined DNA enrichment approach followed by shotgun sequencing to determine the first genome of the first representative of the *Candidatus Parilichlamydiaeae* species, *Ca. Parilichlamydia carangidicola*. The draft genome is 2,089,218 bp long, and contains 1933 predicted coding sequences. Preliminary analysis reveals the presence of a number of conserved proteins, such as structural components of the type three secretion system.

**Conclusions:** The availability of the *Ca. Parilichlamydia carangidicola* genome provides us with new insights into the evolution of members of the order Chlamydiaceae, and can be used to inform future characterisation of novel CLOs.

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**Vancomycin-dependent Enterococcus faecium on screening swabs from a patient on long term oral Vancomycin therapy**

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**Vancomycin Resistant Enterococci (VRE) are an important species well reported nosocomial pathogen which is increasing in incidence worldwide. However, there have only been a handful of case reports describing the isolation of Vancomycin-dependent Enterococci (VDE) on clinical specimens since it was first identified.**

We describe the case of a 54 year old patient with Acute Myeloid Leukaemia and chemotherapy induced neutropenia in whom we isolated VDE on screening swabs. The patient was previously known to be VRE colonised and had an admission with VRE bacteraemia. More recently, the patient had been hospitalised several times with recurrent Clostridium difficile diarrhoea unresponsive to conventional antibiotics. The patient was therefore treated with a prolonged course of oral Vancomycin to offer symptomatic relief.

During the patient’s admission, two routine rectal surveillance swabs for Multi Resistant Organisms (MRO) and VRE screening were performed. As per our laboratory protocol, the swabs were inoculated on to Biomerieux ESBL chromID agar, Thermofisher Scientific HBA + GENT agar, MacConkey agar and VRE chromID agar. We observed significant growth at 48 hours on the VRE chromID agar and the ESL chromID agar. The 3 different colonies isolated were identified by the MALDI-TOF as Enterococcus faecium. All 3 colonies were subsequently subcultured to HBA agar with a Vancomycin disc. Two colonies had a heavy growth resistant to Vancomycin and one of the colonies isolated from the ESL chromID agar grew interestingly only around the Vancomycin disc. Real-Time PCR genotyping of this Vancomycin-dependant isolate identified it as a Van B VDE.

We postulate that the prior and prolonged use of Vancomycin has increased the risk of VDE colonisation in our patient and advocate for greater awareness and testing for this organism in patients at risk.

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**A novel polyphasic framework to resolve Yellow Canopy Syndrome paradox**

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**Objectives:** *Candidatus* Parilichlamydia carangidicola genome provides us with new insights into the evolution of members of the order Chlamydiales, and can be used to inform future characterisation of novel CLOs. The availability of the *Ca. Parilichlamydia carangidicola* genome provides us with new insights into the evolution of members of the order Chlamydiales, and can be used to inform future characterisation of novel CLOs.
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Yellow Canopy Syndrome (YCS) is a largely undiagnosed condition impacting sugarcane crops across Queensland, Australia, causing significant yield losses. YCS is associated with a typical yellowing of cane leaves. Symptomatic leaves senesce and die while the crop can continue to grow. In severe cases, cane stalks become thin and rubbery and root health is compromised. Key YCS symptoms differ from leaf yellowing due to drought stress, phytotoxicity, insect attack, known diseases, nutrient deficiency or natural maturing.

Although some of the above factors have been largely eliminated from further consideration, the causal agent of YCS remains unknown. The aim of this study is to determine the involvement of biotic interactions and soil nutritional health in YCS development.

A comprehensive microbiome-based approach combining Illumina MiSeq and HiSeq and conventional culturing techniques was applied to determine the complexity of organisms present in YCS-affected sugarcane tissues, as well as in rhizosphere soil. Soil physico-chemical analyses, substrate-induced microbial respiration and microbial extracellular enzyme activity measurements were conducted. Salicylic acid content of leaves was determined using HPLC and microscopy-based studies were performed. In addition, qRT-PCR was applied to study the expression of pathogenesis- and stress-related genes in symptomatic vs. healthy sugarcane leaves.

Interestingly, differences were found in bacterial and fungal assemblages of leaves, stalks and roots of asymptomatic vs. symptomatic plants, as well as in the soil nutrient status (C, N) and soil microbial enzyme activity between affected and healthy cane fields. Pathogenesis- and stress-related genes were found upregulated in YCS-symptomatic leaves and symptomatic leaves contained more salicylic acid, demonstrating the potential use of this compound as a biomarker for YCS detection. Microscopic images revealed no blockage or crushing of vessels in YCS-affected sugarcane leaves and roots. Results of this study may reveal the causal agent of YCS and lead to identification of specific management practices for YCS.

Patient shoe covers: transferring bacteria from the floor onto surgical bed sheets

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Introduction

Infection control protocols are followed to prevent environmental contamination by pathogens in operating rooms (OR) to reduce surgical site infections (SSI). Disposable shoe covers used by patients whilst waiting for day surgery, have the potential to contaminate the surgical table if unremoved prior to OR admittance. However, the use and removal of patient shoe covers is not dictated in infection control guidelines. We aimed to determine if disposable shoe covers could transmit bacteria from the day surgery floor to surgical bed sheets.

Methods

Clinical study: Polypropylene disposable shoe covers (n=40) were worn and walking in three locations in day surgery unit: patient waiting area (5 and 10 minutes), toilet (5 minutes) and corridor (5 minutes), on five separate days. Bacterial number and species attached to the shoe covers and subsequently transferred to bed sheets was determined by culture and 16s rRNA sequencing. Laboratory study: The rate of bacterial transmission from artificially contaminated polypropylene and polyethylene shoe covers to bed sheets for the common pathogens Staphylococcus aureus, S. epidermidis, Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecium and Acinetobacter baumannii was determined.

Results

Clinical study: A wide range of bacteria and fungi were found contaminating shoe covers worn in the day surgery unit and in every instance some were subsequently transferred to bed sheets, albeit at a low transfer rate ranging from 1.2% to 5.2%. Staphylococcus species, including S. aureus were the principal bacteria transferred. Laboratory study: For the common pathogens tested, the transfer rate to bed sheets range from 6.9% to 15.1% for polypropylene and 7.5% to 17.7% for polyethylene shoe covers. S. aureus had the highest transfer rate.
Conclusion
We demonstrated that disposable medical shoe covers can transmit bacteria from floor to bed sheets as a potential risk for hospital acquired surgical infections. We suggest an infection control policy should be considered to prevent patients returning to their bed with contaminated disposable shoe covers as this simple measure may reduce theatre contamination and the number of SSI.

Identification of Bacillus cereus emetic toxin gene from Meat Curry
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Bacillus cereus is a spore forming bacterium that produces toxins that cause vomiting or diarrhoea. Symptoms are generally mild and short-lived up to 24 hours. B. cereus is commonly found in the environment as well as a variety of foods. Spores are able to survive harsh environments including normal cooking temperatures. B. cereus is one of predominant food poisoning caused by B. cereus, emetic (vomiting) and diarrheal, have been described in several hospitals in Malaysia. The vomiting type is affected by a small cyclic heat-stable peptide (cereulide), and the diarrheal disease is caused by three different enterotoxins (HBL, NHE, cytK). The objective of this study was to identify B. cereus using biochemical test and emetic toxin-producing of B. cereus, specifically BHL by targeting a specific gene hbd. A total of twenty seven samples of meat curry from nine random restaurants around Selangor, Malaysia were used in this study. Mannitol egg yolk polymyxin (MYP) is a selective medium and confirmatory biochemical tests were used to isolate B. cereus. The biochemical test was used to detect B. cereus and the samples examine for the presence of hbd gene by polymerase chain reaction (PCR). The results showed all samples were identified as B. cereus, the morphology observation showed Gram-positive, rod-shaped and motility differs between the samples. In particular, all samples were positive for the production of the enzyme catalase, fermentation of glucose ,hydrolyzes of starch and the breakdown of amino-acid tryptophan. These findings represent an example of how genomics and biochemical could rapidly help public health experts responding not only to clearly identified select agent but also to agents with similar pathogenic potentials.

A culture-independent comparison of bacterial communities on peripheral intravenous catheters and matching skin swabs
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Pathogenic bacteria present on the skin of a patient are one of the major causes of catheter related bloodstream infections (CRBSIs). The insertion of the catheter makes a break in the skin, and provides direct channel for bacteria to traverse to the blood. Bacteria present on the surface of catheters also form biofilms and can become recalcitrant to antimicrobial therapy. In addition to pathogenic bacteria, the skin harbours a diverse population of non-pathogenic bacteria that can in theory also colonise catheters. These bacteria are typically not identified during routine culture based diagnostic tests performed in clinical microbiology laboratories. In the current study we used culture-independent methods to compare bacterial communities present on peripheral intravenous catheters (PIVCs) with those on a matching skin swab taken the site of PIVC insertion of ten individuals. Our results show that the PIVCs and the skin swabs were colonised with a wide range of bacterial genera, including Methylobacterium, Staphylococcus, Planococaceae group, Pseudomonas, Acinetobacter, Bacillus, Enterobacteriaceae group, Propionibacterium, Corynebacterium and Micrococcus. The Methylobacteria were the most commonly recovered bacteria from both sites. Significantly, the bacterial composition on the skin and PIVC were found to be associated. These findings suggest that the microbiota present on the skin can influence bacterial composition on catheters, and may have implications for both prediction and prevention of CRBSI.

Should we screen women for rectal chlamydia?
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Chlamydia is the most common notifiable Sexually Transmissible Infection (STI) in Australia. Public health control measures rely on screening and treatment of affected individuals and their sexual contacts. The management of rectal chlamydia differs substantially from that of genital/vaginal/cervical chlamydia, making the diagnosis and treatment of rectal chlamydia in at-risk groups a priority. Current screening guidelines recommend rectal-screening for men who have sex with men (MSM), and women with rectal symptoms or reporting anal intercourse, but not for women in general. International studies have reported a prevalence of rectal Chlamydia in women of up to 21% but little data have previously been collected for Australian women. In this study we offered rectal chlamydia Nucleic Acid Amplification Testing (NAAT) to all women attending Canberra Sexual Health Centre (CSHC) from Nov 2013- June 2014 who fell into any of the following three groups: (1) Having symptoms of chlamydia or PID. (2) Presenting as a contact of chlamydia. (3) Attending for followup after a positive urine chlamydia screen. Fifty-seven percent (32/56) tested positive from their rectal sample. There was no association with age, sex, use of sex toys, or rectal symptoms. There was a strong association (p=0.000) with urethral/cervical chlamydia, with 97% (31/32) of women with a positive rectal chlamydia test also having a positive urogenital test. A lack of routine rectal screening in Australia may be resulting in the underdiagnosis and undertreatment of rectal chlamydia, and resultant persistent infection, reproductive tract re-infection, transmission and complications. The high prevalence of rectal chlamydia...
found in this study raises three important questions: Should we be screening women for rectal chlamydia? Should we routinely offer women diagnosed with chlamydia treatment that will effectively treat rectal infection? What is the most effective treatment for rectal chlamydia in women?


5. van Liere GA, Hoebe CJ, Wolffs PF et al. High co-occurrence of anorectal chlamydia with urogenital chlamydia infection in women visiting an STI clinic revealed by routine universal testing in an observational study; a recommendation towards a better anorectal chlamydia control in women. BMC Infect Dis 2014; 14:274-280


1. ACT Government, Holder, ACT, Australia

Ready-to-Eat (RTE) foods have contributed to an increase of food-borne disease outbreaks over the last 20 years as eating out becomes more popular. As part of a food safety monitoring survey in the ACT, 2169 RTE food samples were examined over a 12 year period (2002-2014). This study will summarise the results and look for trends related to the microbiological status of RTE foods for the 12 year period. The RTE food survey examined RTE foods obtained for the following microorganisms; Salmonella spp. Listeria monocytogenes, Bacillus cereus, coagulase positive Staphylococcus and E. coli. Foods were categorised as satisfactory, marginal, unsatisfactory or potentially hazardous depending on microbiological status according to the RTE guidelines issued by Food Standards Australia and New Zealand. Low levels of food-borne pathogenic bacteria were isolated (Salmonella (0.04%), Listeria (2.0%), Staphylococcus (3.0%), B. cereus (6.0%) and E. coli (7.0%)) over the course of the study. Sushi and salad were the two food groups that consistently performed poorly, with 29.0 % and 15.1 % of isolated pathogenic bacteria recovered from these foods respectively. The data shows a generalised trend of improvement. The continuous monitoring and surveillance will provide ongoing information for public health authorities on the microbiological status of ready to eat foods available for purchase in the Australian Capital Territory.

Escherichia coli in poultry meat: prevalence, abundance and phylogenetic profiles

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INTRODUCTION:

Retail poultry meat products are frequently contaminated with E. coli and therefore people may acquire E. coli through ingestion of poultry meat products. E. coli is a common cause of bacterial infection in humans.

OBJECTIVE:

To provide insights into the attributes of E. coli isolated from poultry meat products sold in Australia with special attention to their prevalence, abundance and phylogenetic profiles.

METHODS:

Poultry meat samples were collected during 3 seasons from 16 shops. These shops represented 3 major supermarket chains and an independent butcher that were co-located in the 4 major town centres of Canberra, Australia. E. coli was isolated following enrichment using lauryl sulfate broth or Luria broth plus vancomycin, and also by antibiotic selection. All isolates were assigned to a phylogenetic group using Clermont quadruple PCR and unique strains in a sample were identified by DNA fingerprinting using repetitive element palindromic PCR (REP-PCR).

RESULT:

E. coli was detected in 77.5% of 306 meat samples. Phylogroup A strains were most common among the 3415 E. coli isolates, with phylogroup B2 strains being the least common. The B2 strains are potential human ExPEC (extraintestinal pathogenic E. coli) strains while A strains are typically over-represented in live chickens. Identical strains were observed from different meat types, retailers, and geographic locations. Poultry meats from

Trends in the microbiological status of ready-to-eat foods in the Australian Capital Territory from 2002-2014

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independent butchers were less likely to have E. coli than the major retailers. Gizzards of whole meat had higher incidence of E. coli than mince, breast or wings. Products from organically reared or conventionally reared chickens were more likely to have E. coli than products from free range chickens. E. coli detection was significantly high during summer than autumn but intermediate in winter.

CONCLUSION:

The results of this study provide further evidence that the food we consume is a significant source of E. coli. The results also suggest that some post processing contamination of poultry products may be occurring.

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Presence of Escherichia coli environmental clades within B1 phylogroup isolated from water catchments in Eastern Australia

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Escherichia coli is widely used as an indicator of faecal contamination in water, as it is easily identified, a ubiquitous inhabitant of the intestinal tract of humans and other mammals, and believed to persist for only a few days once outside the host.

Strains of E. coli can be partitioned into subgroups that have been denoted as A, B1, B2, D, C, E, F and clade I-V. The clonal composition of E. coli inhabiting most host populations does not reflect the clonal composition of E. coli isolated from water bodies. In water bodies, strains belonging to phylogroup B1 predominate. Previous studies have suggested that some B1 strains may have enhanced persistence in water (>12 days) relative to other E. coli strains. Although B1 strains are uncommonly detected in human faeces, they are very common in some host groups, such as carnivorous mammals and birds.

A recent study demonstrated that at least one lineage of B1 strains (clonal complex B7) represents a predominately host associated lineage. However, the relative abundance of this lineage in water is unknown, nor is it known if there are B1 strains predominately associated with non-host environments. Consequently whole genome sequencing was undertaken on a collection of 116 B1 strains that had been repeatedly isolated from water bodies across eastern Australia, together with 76 diverse faecal B1 strains.

Phylogenetic analysis revealed two distinct lineages of B1 strains that predominately consisted of environmental isolates. These lineages lack antibiotic resistance determinants and harbour very few virulence factors associated with either intestinal or extra-intestinal disease. Consequently, these strains appear to be members of autochthonous populations of E. coli that inhabit diverse water bodies.

This study enhances our understanding of persistent B1 E. coli strains in water and demonstrates that their existence further confounds E. coli’s use as a water quality indicator of faecal contamination.

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Detection of hazardous analytes in the environment using electroactive bacterial biosensors: A synthetic biology approach

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Detection and quantification of a wide range of hazardous substances (e.g. heavy metals) is required to minimise harm to the ecosystem of contaminated environments. Analytes of these hazardous substances enter and accumulate in the food chain causing potential harm to humans. Current detection methods of contaminants in water and soil environments are not always practical as they are time-consuming, costly and require off-site testing. These limitations can be overcome using whole cell biosensors. The ability to genetically manipulate regulatory elements to produce a detectable and measurable signal with a range of sensitivities and specificities has resulted in the utilisation of commonly used laboratory microorganisms as biosensors. For whole cell biosensors to be feasible for the detection of contaminants in the environment, a wider range of microorganisms with integrated output systems is required. This study is focused on utilising electroactive bacteria (Pseudomonas, Shewanella and Geobacter) as biosensors. These microorganisms can interact directly with electrode surfaces and have the potential to be integrated into electronic devices. Redox-active proteins which are produced by these bacteria have specific electrochemical signals that can be detected using cyclic voltammetry. As a proof of principle in the development of integrated biosensors, genes encoding electroactive cytochromes have been cloned downstream of promoters and their cognate electroactive bacteria into electronic devices. Redox-active proteins which are produced by these bacteria have specific electrochemical signals that can be detected using cyclic voltammetry. As a proof of principle in the development of integrated biosensors, genes encoding electroactive cytochromes have been cloned downstream of promoters and their cognate electroactive cytochromes have been cloned downstream of promoters and their cognate electroactive cytochromes have been cloned downstream of promoters and their cognate electroactive cytochromes have been cloned downstream of promoters and their cognate electroactive cytochromes have been cloned downstream of promoters and their cognate environmental contaminants.

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Synthetic biology derived biosensors for heavy metal detection

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Anthropogenic activities have led to increased environmental contamination, resulting in a need for constant monitoring of dangerous analytes. Heavy metal contamination is of particular concern to human health as they are non-biodegradable and retained within ecological systems. Synthetic biology offers the ability to repurpose microbial genetic responses to heavy metals, enabling development of heavy metal microbial biosensors.

Utilising synthetic biology capabilities, de novo biosensing constructs have been developed for arsenic, cadmium, chromium, lead, mercury and zinc, for detection of heavy metal ions at levels deemed dangerous by the World Health Organisation to human drinking and ground water. Synthetic pathways have been developed consisting of a modular input combined with a detectable output. In these systems, an increase of heavy metal ions into the bacterial cell will
result in the expression of a fluorescent protein allowing both qualitative and quantitative analysis. Initially, designs were focused on single-input/output constructs; however the development of multiplexed biosensors that can detect multiple inputs and/or provide multiple outputs will allow more dynamic sensing.

These biosensing constructs have been developed such that they are transferable between a broad range of environmentally relevant microbes including Shewanella oneidensis, Pseudomonas and Bacillus spp. To expand this range further, and mitigate the deleterious affect of the heavy metals, microbes isolated from known contaminated environments, with a higher tolerance towards heavy metals, are being utilised. As a result whole cell biosensors can be produced with sensing capabilities in a broader range of environments.

Using defined input and output modules along synthetic biology principles is allowing the targeted development of a range of microbial biosensors to analytes in the environment. Future developments of these sensors will allow detection of a wider range of contaminants in soil and aquatic environments leading to long-term monitoring devices allowing real-time analysis.

Expression of human fucosyltransferase 3 (FUT3) in Trichoderma reesei
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Glycan-modifying enzymes are increasingly sought after for in vitro glycan analysis and modification. A bottleneck limiting progress in the glycobiology research is the non-availability and/or high price of glycan-modifying enzymes on the market.

The broad aim of this project is to produce recombinant fucosyltransferases of human origin in Trichoderma reesei, a high-yielding fungal cell factory. T. reesei is a eukaryote with a well-developed posttranslational protein processing machinery featuring glycosylation, phosphorylation and disulﬁde bond formation. In addition to a long history of safe industrial use and ability to express heterologous proteins, T. reesei is also easier and cheaper to cultivate on a large scale than mammalian or insect cells typically employed for the production of recombinant gene products of human origin.

In the current work, cDNA encoding the C-terminal catalytic domain of the human α-1,3/4 fucosyltransferase (FUT3) was synthesised according to the T. reesei codon usage and assembled into an expression vector under the strong cellobiohydrolase 1 promoter. The expression design allowed FUT3 to be produced as a fusion to the core domain of the human hepatocyte growth factor receptor (α1-HGF). The sized fusion did not only demonstrate secretion of a full-sized fusion protein but also degradation of the heterologous protein, most probably by the remaining Trichoderma proteases. The time- and pH-dependent degradation of the recombinant FUT3 makes it necessary to optimize the cultivation conditions in order to obtain sufﬁcient amounts of the protein for puriﬁcation, biochemical characterisation and determination of the nature of the degradation products.

Evolving Resistance to Imatinib: From Molecules to Mice
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Global incidence of resistance to current antimicrobial drugs is on the rise, highlighting the urgent need to develop novel antimicrobials. Antimicrobials currently in use typically target pathogen encoded proteins or pathways, leading to selective pressure to acquire resistance. Resistance mechanisms, both acquired and innate, are generally elicited by the cellular stress response. A recent approach targets host factors that the invading microbes rely on for their spread, virulence or survival. It is proposed this approach will produce antimicrobials that are much less likely to select for resistance. This study uses vaccinia virus (VACV) to model the development of resistance to host targeted antimicrobial imatinib, which inhibits the release of enveloped virus.

Previous work has isolated a putative imatinib resistance allele through culturing virus in the presence of imatinib. A mutation in this isolate was identiﬁed in the outer envelope viral protein A34. This study aims to characterise the novel A34 mutation (A34K111T) as well as another published mutation in A34 (A34I120D), both leading to higher virus release from cells than the parental WR strain. Results show that the two alleles in A34 resist imatinib treatment to a differing extent, and potentially represent different modes of resistance. This study has also ampliﬁed and cloned the A34K111T point mutation into a plasmid vector, and used homologous recombination to insert the mutation back into the parental background, laying the foundation for creation of a recombinant virus to further characterise the allele. A putative recombinant awaits sequencing and characterisation. As well as in vitro characterisation of the viral strains mice will be used for in vivo studies. Mice infected with a fatal dose of VACV survive when treated with imatinib. In vivo studies will establish whether these mutations in A34 restore fatality in infected mice when treated with imatinib.

Comparative analysis of koala retrovirus (KoRV) pathogenesis between Queensland and South Australian koalas
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Retroviral particles were first identified in tissues from a leukaemic koala in 1988. The virus was successfully isolated from Queensland (QLD) koalas and the full genome was sequenced in 2000. Koala retrovirus (KoRV) is the only known exogenous retrovirus undergoing a process of active endogenisation in its host at the present time. KoRV is 100% prevalent within QLD koalas and the virus has endogenised in that population, with high proviral (DNA) loads reported. These populations also have high levels of chlamydiosis (40%) and neoplasia. The scenario is different in South Australia (SA) where KoRV-free animals are present, and apparently both endogenous and exogenous forms of the virus exist. There is anecdotal evidence that the picture of population disease associated with KoRV infection differs between these two geographic areas however the source and relevance of these differences is unknown. The aim of this project is to characterise these differences and to explore possible reasons, whether they are host or viral factors. Viruses from both areas will be characterised, proviral and viral (RNA) load will be quantified and compared to pathology and the host response to infection will be characterised using transcriptome analysis. It is hypothesised that high viral load is associated with disease advancement because, as the viral load becomes high, the chance of insertional mutagenesis and subsequent tumour induction increases. Virus will be cultured to investigate any sequence variation between virus isolates from SA and QLD koalas. Transcriptome analysis will identify differences in expression of genes associated with the immune response between QLD and SA populations and between KoRV-infected and uninfected koalas. The transcriptome study may also provide information about characteristics of KoRV in the koala genome. Preliminary results will be presented. It is anticipated that this information will identify factors that influence development of KoRV associated disease, information that can hopefully be used to design koala management programs and disease prevention measures.

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Molecular diversity of Staphylococcus aureus from dairy farms in the State of Victoria

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Pathogenic strains of Staphylococcus aureus can cause disease in both humans and animals. In animal species, including ruminants, S. aureus is capable of causing severe mastitis and sub-clinical mastitis. Animals with mastitis frequently shed S. aureus into the milk supply which can result in food poisoning in humans. The aim of this study was to use typing methods including pulsed field gel electrophoresis (PFGE), multi-locus sequence typing (MLST) and enterotoxin profiling to understand the diversity of S. aureus isolates from seven dairy farms in Victoria. A survey of 120 samples collected from three bovine, two caprine and two ovine dairy farms yielded 13 isolates of S. aureus from milk and milk filter samples. Caprine and ovine isolates shared greater than 80% similarity regardless of source using PFGE. Conversely, bovine isolates were shown to be of much greater diversity. MLST of the bovine isolates found sequence types (STs) of both human and ruminant origin. In contrast, all caprine and ovine isolates belonged to ST133, this has been observed in small ruminants from other countries. Staphylococcal enterotoxin (SE) profiling indicated caprine and ovine isolates possessed 1-3 different SEs, whereas bovine isolates carried between 1-9 SEs. This study indicates that S. aureus from ruminant animals are varied; however, dominant STs may exist within particular animal types. Gaining an understanding of the genotypic diversity that exists within Australian S. aureus isolates may assist the development of future treatment strategies.

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Unveiling the microbiome of Australian ticks

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Ticks transmit a greater variety of pathogenic microorganisms than any other arthropod group and are among the most important vectors of diseases affecting humans, livestock and companion animals. The huts, hence the components of the tick microbiome have been studied extensively. In this study, microbial communities were characterised from the Australian paralysis tick I. holocyclus and the European sheep tick I. ricinus. Initial analysis identified that ~99% of 16S sequences from I. holocyclus and I. ricinus ticks belonged to the tick intracellular endosymbiont Candidatus Midichloria mitochondrii. Relative copy-number of the Midichloria masked other bacterial communities and was impervious to switching 16S primers, sequencing platform or sequencing at greater depths. The development and use of a specific Candidatus M. mitochondrii blocking primer decreased the number of endosymbiont sequences by 95.8% in I. holocyclus and 96% in I. ricinus samples. This allowed identification of 199 and 94 bacterial genera in I. holocyclus and I. ricinus ticks, respectively. Of particular interest, a novel Candidatus Neoehrlichia sp. was identified in I. holocyclus. Recently, Candidatus Neoehrlichia mikurensis was identified as an emerging tick-borne pathogen of animal and human health importance. Our preliminary data, with a 94-95% sequence homology to Candidatus Neoehrlichia spp., indicates an Australian variant found within I. holocyclus. These results show that without blocking primers and the use of NGS molecular advances, many bacteria of potential medical and veterinary interest could go undetected.

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Defining core genome of Salmonella Typhimurium for epidemiological typing

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Salmonella enterica serovar Typhimurium is the most common serovar in both human infections and farm animals in Australia and many other countries. High typing resolution is critical for the detection of outbreaks and the investigation of within-patient variation in clinical samples. The combination of SNP typing and next generation genome sequencing opens a new avenue for the application in diagnostic and public health microbiology. In this study, 62 S. Typhimurium genomes were used for identification of the core genome content. The assembled genomes were...
aligned to the reference strain LT2 using the progressiveMauve. 3846 genes and 1576 intergenic regions were defined as the Typhimurium core genes and proposed for outbreak detection. 45.5% Typhimurium genomes from eight outbreaks were analyzed, including seven strains from HIV-infected adults outbreak in Malawi from 2002 to 2008, 12 isolates from outbreak occurred in Tasmania between 2005 and 2008 and isolates from six outbreaks that derived from the Danish laboratory based surveillance system of human gastrointestinal infections in 2000–2010. SNPs in core genome of S. Typhimurium were extracted on the basis of their genome sequences. The results showed that SNP-free was sensitive to the outbreak detection, which achieved 100% separation between outbreak isolates, even for the subclade of outbreak isolates. This approach is also likely to be useful for other pathogenic bacterial species, providing a more reliable marker for epidemiological analysis.

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Emergence of a New Lineage of Dengue Virus Type 2 Identified in Travelers Entering Western Australia from Indonesia, 2010–2012

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Dengue virus (DENV) transmission is ubiquitous throughout the tropics with the Asia-Pacific region responsible for greater than 70% of the current global dengue disease burden. DENV serotype and genotype data is lacking in many parts of this region, limiting our attempts to understand the observed patterns of hyperendemicity and disease severity.

Many countries in the Southeast Asia and Western Pacific region are popular tourist destinations, and dengue has been identified as a cause of travel-related illness in people returning from endemic countries. We sequenced the E gene of DENV isolated from travellers returning to Western Australia from 7 countries throughout Asia between 2010 and 2012. We identified a diverse array of multiple co-circulating DENV-4 viral lineages with the majority originating in Indonesia, predominantly Bali, a popular travel destination for Australians. Most viruses were closely related to lineages known to have circulated in Indonesia for some time, indicating that this geographic region serves as a major hub for dengue genetic diversity. Most interestingly, we identified a new lineage of DENV-2 (Cosmopolitan genotype) that emerged in Bali in 2011–2012 which should be monitored. Travellers may act as sentinels and provide important information on DENV genotypes and lineages circulating in countries where locally generated detailed genetic data may not be available.

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The prevalence of Angiostrongylus mackerrasae/cantonensis complex in the greater Sydney region

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Angiostrongylus cantonensis and Angiostrongylus mackerrasae are parasitic nematodes found in various rat species. In humans A. cantonensis causes the disease angiostrongyliasis which is the most common cause of eosinophilic meningitis worldwide, while A. mackerrasae has shown incidence of disease in animals. Intermediate hosts of these nematodes include a diverse range of molluscs (mainly slugs and snails) which can maintain the infective L3 larval stage of the parasite. Humans, and other large vertebrates are dead-end hosts which become infected following ingestion of molluscs, paratenic hosts or contaminated produce containing infective A. cantonensis larvae. In Australia, recent clinical cases of angiostrongyliasis have been reported in Queensland and New South Wales indicating the need for further research to elucidate the risk of angiostrongyliasis in this region.

In this study, a conventional PCR and a TaqMan assay were compared for their ability to detect Angiostrongylus DNA in DNA extracted from molluscs. Both assays amplified A. cantonensis and A. mackerrasae DNA though the TaqMan assay was more sensitive, detecting an equivalent to one hundredth of a worm larvae. Subsequently, the TaqMan assay was employed for field testing. Sequencing of the 185 RNA and ITS1 genes of the two Angiostrongylus species revealed little to no difference at these loci (99% identical). A total of 500 molluscs were collected from the Sydney region for DNA extraction. The TaqMan assay detected Angiostrongylus DNA in 3.0% ±0.8% (CI 95%) of molluscs surveyed. This is the first Australian study to survey molluscs using PCR and is the first to report a natural reservoir for Angiostrongylus spp. in Cornu aspersum, which is now thought to be a common intermediate host for Angiostrongylus spp. in Sydney. This study confirms the presence of the A. cantonensis/mackerrasae complex in Sydney and identifies a ubiquitous natural reservoir (C. aspersum) of A. cantonensis in the area.

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The survival mechanisms of Vibrio cholerae in the presence of protist grazers; a model for understanding cholera and other V. cholerae related disease.

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Cholera kills approximately one hundred thousand people every year with the global price tag of combatting the spread of this devastating disease exceeding hundreds of
Human rhinovirus 3C protease cleaves RIPK1, an important intermediate in extrinsic apoptosis
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Human Rhinovirus (HRV) is a human pathogen of significant medical importance, being a major cause of upper respiratory tract infections and causing majority of the virus-induced asthma exacerbations. In this study, we investigated whether HRV could regulate apoptosis, a key antiviral innate immune response. The apoptotic signal is generated either via receptors (extrinsic) or disruption of homeostasis within the cell (intrinsic) and is propagated via caspase cascades that lead to cell death, thereby reducing HRV viral replication which relies on cellular machinery.

We have used HRV16 (a group A HRV that uses ICAM-1 as receptor) infected cells, cells treated with chemical inducers and inhibitors of extrinsic apoptosis, and in vitro protease cleavage assays to show that HRV16 3C protease cleaves a key intermediate in extrinsic apoptosis, RIPK1, an extrinsic apoptosis adaptor protein, was cleaved by caspase 8, as expected, during chemical induction of extrinsic apoptosis. RIPK1 was also cleaved in HRV infection albeit at a different cleavage site. Interestingly, caspase 8 activation, which is associated with extrinsic apoptosis, was required for optimal HRV 3C protease mediated cleavage of RIPK1. This was potentially achieved by increasing the accessibility of the HRV 3C cleavage site within RIPK1. The caspase 8 mediated RIPK1 cleavage product has a pro-apoptotic function, and further cleavage of this pro-apoptotic cleavage product by HRV 3C may provide a mechanism by which HRV regulates apoptosis.

Understanding the interaction of the pathogenic intracellular bacterium Coxiella with the mammalian host: A metabolomics perspective
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Coxiella burnetii is an intracellular bacterium responsible for the zoonotic disease Q fever. A recent outbreak in the Netherlands cost over 300 million Euros to control, and resulted in long-term ill health in some patients. Until recently Coxiella was considered an obligate intracellular organism, hampering attempts to characterise its pathogenic mechanisms. The publication of axenic culture methods in 2009 facilitated the development of genetic manipulation methods for Coxiella, and has now enabled us to optimise metabolome study to apply metabolomics to Coxiella. This allows us to both dissect its central carbon metabolism, potentially identifying essential pathways and substrates, and to use metabolomics as a tool for functional characterisation.
of genes we have identified as required for intracellular growth. Following optimisation of quenching and metabolite extraction techniques, we compared the metabolite profiles of 6 day cultures, representing the replicating form of the bacteria (large cell variant; LCV), with the profiles of 20 day cultures that represent the non-replicating form of the bacteria (small cell variant; SCV). Historically, SCVs are considered to be metabolically inactive, and multivariate analysis revealed differing overall metabolic profiles for LCVs and SCVs, with specific differences in metabolites from glycolysis and the tricarboxylic acid (TCA) cycle. Interestingly, 13C-glucose labelling experiments demonstrated labelling of glycolytic and TCA cycle intermediates in both culture stages, suggesting that SCVs are metabolically active. Analysis of the metabolite profiles of wild type Coxiella and the cbu_0364 mutant, which lacks a gene encoding a putative transporter and is attenuated for growth inside mammalian cells, revealed a number of significantly different compounds. This will inform future transport assays to elucidate the function of CBU_0364. Our work is the first application of metabolomics technology to understanding Coxiella pathogenesis, and opens up many new avenues for investigation.

### The double burden of diabetes and bacterial infection - a model to investigate co-morbid disease

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**Introduction:** Type 2 diabetes (T2D) is a chronic inflammatory condition which induces immune dysfunction through uncontrolled hyperglycaemia and subsequent oxidative stress. Low- to middle-income countries with high burdens of infectious diseases contribute to >80% of the T2D pandemic. Patients with T2D have a significantly increased risk of acquiring intracellular bacterial infections such as tuberculosis (TB) and melioidosis. In tropical Australia co-morbid diabetes was reported in 23% of patients with TB. Whilst this association has been recognised, the host-pathogen interactions contributing to susceptibility remain poorly defined, with current in vivo models inadequate to investigate co-morbid infection.

**Methods:** This research involved the development of the Diet-Induced Diabetes Co-Infection (DID) murine model, to reflect the pathognomonic characteristics of human T2D. Male C57BL/6 mice were fed a high fat, high glycaemic index (HFHG) diet and the biochemical changes, inflammatory status and immune response when challenged with intracellular bacterial pathogens determined over 30 weeks.

**Results:** Mice on the HFHG diet demonstrated the cardinal signs of T2D, including glucose intolerance, hyperglycaemia and advanced glycation end product formation. Furthermore, 87.0% of mice (n=20) on the HFHG diet developed dyslipidaemia and evidence of renal impairment, a leading complication associated with T2D. When infected subcutaneously with Burkholderia pseudomallei NCTC 13178 (9x10^15 CFU), an intracellular bacterial pathogen, mice on the HFHG diet had a significantly higher bacterial burden in the spleen and blood and succumbed to infection more rapidly compared to control animals. The HFHG diet also contributed to baseline immune dysregulation and impaired cytokine responses following infection challenge.

**Conclusion:** The development of the DIDI model has facilitated co-morbid infection studies that better reflect the intrinsic host-pathogen interactions involved in the increased susceptibility of patients with T2D.

### Pentraxin 3: A key player in shaping arthritogenic alphavirus disease pathogenesis

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**Introduction**

The rising prevalence of arthritogenic alphavirus infections including chikungunya virus (CHIKV) and Ross River virus (RRV) and the lack of antiviral treatments highlight the potential threat of a global alphavirus pandemic. The immune responses underlying alphavirus virulence remain enigmatic. Herein, the role of pentraxin 3 (PTX3), an acute phase innate immune protein, in facilitating alphaviral disease progression was characterised.

**Methods**

PTX3 expressions in PBMC and serum specimens of CHIKV-/RRV-infected patients were determined using qRT-PCR and ELISA, respectively. PTX3-deficient (PTX3−/−)/wildtype [WT] C57BL/6 mice were infected with RRV and monitored for disease. Profiles of immune mediators were determined using qRT-PCR. HEK293T cells were transiently transfected with PTX3 plasmids prior to RRV/CHIKV infection. Virus burden were determined using plaque assay and viral load qRT-PCR.

**Results**

PTX3 was highly expressed in CHIKV and RRV patients during acute disease. Overt expression of PTX3 in CHIKV patients was associated with increased viral load and disease severity. RRV-infected PTX3−/− mice exhibited delayed disease progression and rapid recovery through diminished inflammatory responses and viral replication. Transient overexpression of PTX3 in HEK293T cells resulted in enhanced viral entry and replication during RRV/CHIKV infection, leading to high viral burden. N-terminus of PTX3 was identified to be the functional domain modulating alphaviral infection.

**Conclusion**

This study demonstrates a previously undescribed pivotal role of PTX3 in shaping alphaviral disease progression through immunomodulation and facilitating viral infection and replication processes during the acute infection. Understanding the complex immune responses elicited during alphaviral infection will be integral for future development of therapeutic interventions.
The role of intracellular cholesterol within macrophages exposed to *Mycobacterium avium* subsp. paratuberculosis

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Johnne’s Disease (JD) is a chronic granulomatous enteritis caused by *Mycobacterium avium* subspecies paratuberculosis (MAP), primarily affecting a wide variety of agriculturally significant ruminant species such as sheep, cattle, deer and goats. Following ingestion, MAP is translocated across the intestinal epithelium via specialised M cells located in the Peyer’s patches and into the intestinal submucosa whereby MAP localises within host macrophages upon phagocytosis. In recent years, there have been numerous studies that have identified cholesterol as a key factor influencing the establishment of mycobacterial infection within macrophages through prevention of phagocyte maturation. Additionally, cholesterol has been recognised as a major energy source that can be utilised by mycobacterial species. However, despite these implications of cholesterol associated with MAP, the mechanisms driving these cellular alterations are yet to be fully understood. The main objective of this study was to determine if there is a colocalisation of cholesterol with GFP-labelled MAP within macrophages following exposure to MAP. Using fluorescent microscopy, we observed that there was a noticeable redistribution of cholesterol within macrophages exposed to MAP. Furthermore, in macrophages exposed to MAP there was an accumulation of intracellular cholesterol at focal points located throughout the cell. In addition, the expression of cholesterol associated genes was explored in this study to further understand the relationship between MAP and the host. The findings from this study may represent a mycobacterial adaptation to evade detection during the early stages of infection.

Preterm infants and cytomegalovirus in breast milk: an ordinary virus in an extraordinary setting.

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Around 50–70% of Australian women will have already been infected with cytomegalovirus (CMV) when they become pregnant. It is a little known fact that the majority of these women will naturally excrete cytomegalovirus into their breast milk and that a proportion of babies will become infected though milk ingestion. Although this is generally of no medical concern, in the extraordinary setting of the neonatal intensive care unit, cytomegalovirus infection can be difficult to diagnose and to manage. Common symptoms are hepatitis and sepsis like syndromes and the long term consequences of infection for later developmental outcomes are not clear.

We collected breast milk from 29 mothers of very preterm babies (<32 weeks gestation) on days 2-4, 8-12 and 26-30 post partum and evaluated the milk for the presence of CMV by PCR analysis and long term culture. We also evaluated breast milk and maternal plasma for the presence of CMV-specific IgG. Whilst maternal plasma was not a useful indicator of viable CMV in breast milk, higher titres of CMV-specific IgG in breast milk were associated with viral isolation from samples collected at later times post partum. In most cases these samples were detected by quantitative PCR, but the virological copy number was very small. Other possible biological indicators of infection such as the presence of cytokines and other immunomodulatory factors such as lactoferrin and lysozyme were also assessed, but no other correlates to potential infection were determined.

Although 60% of women were positive for CMV (determined by maternal plasma titre and CMV-specific IgG present in breast milk and/or PCR positive breast milk or CMV detected in culture), only one baby was determined to have clinically significant CMV infection. This underscores the difficulty with evaluating post-partum infection in this setting, and suggests that any interventions will need to be well justified and carefully evaluated.

Characterisation of a family of novel glycosyltransferase effectors from *Salmonella*

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Pathogenic serovars of *Salmonella* are the causative agents of a variety of disease states, including typhoid fever, self-limiting gastroenteritis, and invasive bacteremia. Pathogenesis is dependent on the activity of two distinct type III secretion systems, encoded by genetic regions termed *Salmonella* pathogenicity islands (SPI). Effector proteins secreted by SPI-1 play important roles in colonisation of the gastrointestinal tract, while effectors of the SPI-2 system are involved in intracellular persistence, and the establishment of the *Salmonella*-containing vacuole (SCV), a critical replicative niche. However, many of the SPI-2 encoded effector proteins remain poorly characterised. Our work focuses on the SseK family of SPI-2 effector proteins, consisting of SseK1, SseK2 and SseK3. The SseK family is homologous to the NeiB effector from enteropathogenic *E. coli*, which was recently reported to be a novel glycosyltransferase that adds a single N-acetylglucosamine to human FADD, blocking the host apoptotic pathway. We predict the SseK proteins have similar enzymatic activity, however we have established that FADD is not the target. Therefore, the aim of our work is to characterise the role of the SseKs in *Salmonella* infection, and in particular to identify putative targets from the host cell. We describe here the subcellular localisation of the SseK proteins in HeLa cells in both transfection and infection experiments. SseK1 predominantly localises to the nucleus, while SseK2 and SseK3 localise predominantly with the Golgi apparatus, suggesting that these proteins may have some interaction with host Golgi proteins. Additionally, SseK3 extends along the *Salmonella*-induced filament structures and cells of this phenotype have disrupted Golgi apparatus. These preliminary data show the SseK proteins have different subcellular localisation phenotypes, and may suggest possible roles in *Salmonella* infection. We intend to advance the understanding of the SseK proteins by identifying binding partners through immunoprecipitation, mass spectrometry and yeast two-hybrid screens.
The characteristics of genetically related *Pseudomonas aeruginosa* from diverse sources and their interaction with human cell lines

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A collection of 234 *Pseudomonas aeruginosa* strains isolated from hospitalized patients (n=20), inlet effluent of a local sewage treatment plant (STP) (n=136) and from six sampling sites of a river (n=78) downstream of the STP, were tested for their clonality using RAPD-PCR, the presence of seven virulence genes (VGs), their resistance against 12 antibiotics and their adhesion to three human cell lines. A high diversity was found among the isolates irrespective of their sources with only six common (C) types containing strains from clinical and environmental sources. Environmental strains belonging to these C-types showed a higher level of adhesion to A-498 cells than clinical strains (17±13 versus 13±11; p=0.001), whereas clinical strains adhered significantly greater to Calu-3 and Caco-2 cells than environmental strains (p<0.001 for both). The VGs and antibiotic resistance profiles of the strains were similar, however the prevalence of STP strains carrying both exoS and exuO was significantly (p=0.0368) higher than clinical strains. Whilst all strains were resistant to ticarcillin and ticarcillin/clavulanic acid resistance against aztreonam (98%), gentamicin (39%), amikacin (16%) and ceftazidime (8%) were higher among river than STP and clinical strains. In contrast resistance against piperocillin was higher amongst clinical strains (57%) than STP (27%) and river (16%) strains. These results suggest that environmental strains of *P. aeruginosa* carrying similar virulence properties including adhesion to various human cell lines which makes them capable of causing infection in humans under predisposing conditions.

Plaque-to-plaque bottleneck passage of dengue viruses leads to fitness loss and extinction.

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Muller’s Ratchet is the process by which asexual populations lose fitness when subjected to repeated genetic bottlenecks[1]. Similar bottlenecks may be common during transmission of arboviruses as they move between insect and vertebrate hosts. To determine the effect of Muller’s Ratchet on transmission of dengue viruses (DENV), progeny from 10 plaque purified populations of DENV derived from an infectious cDNA clone were subjected to repeated plaque-to-plaque passages. Although eight of the 10 clones were able to produce viable progeny after 10 plaque-to-plaque transfers, one no longer produced plaques after 3 and another after 4 passages and competition assays demonstrated that all 10 single plaque populations had lost fitness and plaque-to-plaque transfers lead to further fitness loss. Analyses of the envelope (E) genes of these populations found that the genetic diversity of the single plaque virus was reduced by more than ninety per cent compared to the parental population from which they were derived and that the repeated plaque-to-plaque transfer also led to a change in the consensus sequence of the populations. These results demonstrate that Muller’s Ratchet operates on populations of dengue virus during repeated bottleneck passages and that it may lead to their extinction.
Re-emergent enterovirus 71 genotype B5 in 2012 belongs to a new sub-lineage of B5 with predominate substitutions in 3D polymerase and higher replication rate

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Clinical manifestations of enterovirus 71 (EV71) range from herpangina, hand-foot-and-mouth disease, to severe neurological complications. Instead of switching genotypes seen in EV71 outbreaks during 1998-2008 in Taiwan, genotype B5 was responsible for two large outbreaks in 2008 and 2012. To examine whether EV71 strains from both genotype B5 outbreaks were the same, we analyzed the full-length sequences of isolates from Taiwan. EV71 strains from both outbreaks were phylogenetically segregated into two lineages containing fourteen non-synonymous substitutions in non-structural protein coding region predominantly; interestingly, seven were in 3D polymerase region. We compared B5 viruses of each from 2008 and 2012, and found 2012-EV71 had higher growth kinetics. We then constructed reverse genetics (rg) viruses containing 3D-2008 and 3D-2012-protein region, respectively, by using N7008TW99 (genotype B4) as the backbone. 3D-2012 rg virus showed faster replication rate than 3D-2008-rg virus, which was similar to native viruses. These findings suggest that the 3D protein region important for viral polyomtivation activity and replication play a role in displaying differential properties between 2008 and 2012 strains. The results indicate re-emergent genotype B5 in 2012 belonged to a new sub-lineage of B5 with higher replication than 2008 B5 strains, which may contribute to the large EV71 outbreak in 2012 in Taiwan.

A novel capsular typing method for Streptococcus pneumoniae using Minimum SNPs

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Purpose of Study

Streptococcus pneumoniae, a potentially deadly bacterium, has the ability to switch its protective polysaccharide capsule via the process of horizontal gene recombination. Termed “capsule switching”, this is problematic globally since this bacterium can evade the current pneumococcal vaccines (1). Determination of these capsule switches largely relies on the combination of traditional serotyping methods and bacterial fingerprinting methods e.g. Pulsed Field Gel Electrophoresis (PFGE) and Multi-Locus Sequence Typing (MLST). Serotyping and whole capsule sequencing are laborious and expensive. Multiplex PCR reactions have been developed for capsule identification but none have been tested on all 98 possible capsule types (2). This study aims to develop a novel capsule typing method applicable to all pneumococcal serotypes using a bioinformatics approach to identify polymorphic genes within the capsule sequence. Used in combination with a bacterial fingerprinting method, capsule switching could be determined.

Method

A total of 93 available S. pneumoniae capsule sequences from NCBI database were analysed using a Minimum SNPs bioinformatics program (3). The program could identify a minimum number of capsule genes that would characterise each capsule type. The capsule typing method was run in silico and then PCR was performed to demonstrate the application to Queensland clinical isolates of S. pneumoniae.

Results

In silico data analysis identified 28 capsule genes that could distinguish 34 serotypes, and 39 serogroups (Simpson’s Index of Discrimination = 0.9883). For Queensland, only 17 capsule genes were required to distinguish all the serogroups and 21 serotypes (out of 35 serotypes detected in Queensland). PCR demonstrated the absence/presence of the capsule genes which can be used to display a capsule type profile.

Conclusion

Identification of pneumococcal capsule switching is important, particularly in light of vaccine evasion. Our study has demonstrated differentiation of the majority of pneumococcal serogroups using a bioinformatics approach. The potential for this inexpensive and quick capsule typing method may enable the rapid detection of pneumococcal serotypes and capsule switching events worldwide.

Microevolution of epidemic Australian Bordetella pertussis isolates

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Despite high pertussis vaccination coverage, Australia experienced a prolonged epidemic in 2008-2012. The predominant B. pertussis genotype harboured pertussis toxin promoter allele, pfxP3, and pertactin gene allele, pm2. The emergence and expansion of non-expressing pm isolates (Pm negative), was also observed. In this study, whole genome sequencing was used to investigate the evolution and genomic diversity of 22 epidemic B. pertussis isolates, including 10 Pm-negative strains with three different modes of inactivation (IS481F, IS481R and IS1002). Five pre-epidemic strains were also sequenced for comparison. Five single nucleotide polymorphisms (SNPs) were common in the epidemic isolates and differentiated them from pre-epidemic isolates. We found that virulence-associated genes had higher numbers of non-synonymous SNPs, indicating selection pressure driving their evolution and that isolates not expressing Pm were derived independently multiple times. We conclude that SNPs play an important role in the adaptation and microevolution of the 2008-2012 epidemic B. pertussis.

RHD-Accelerator: a pipeline for directed evolution of Rabbit haemorrhagic disease virus to generate antigenic variants.

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Rabbit haemorrhagic disease virus (RHDV) is a positive sense RNA virus in the family Caliciviridae. It causes a necrotising hepatitis in European rabbits (Oryctolagus cuniculus), with a mortality rate of approximately 90% in susceptible adult rabbits. In Australia, the RHDV Czech strain V-351 has been widely used as a rabbit biocontrol agent since its release in 1995. However, the current effectiveness of this strain in the field is limited by widespread immunity, and also by emerging genetic resistance. This study aims to develop an experimental platform for the isolation and characterisation of antigenically novel RHDV strains for ongoing sustainable rabbit biocontrol in Australia. As RHDV does not replicate in cell culture, antigenic variants were selected by serially passaging a highly virulent RHD field isolate in laboratory rabbits that had been passively immunised with increasing monoclonal antibody concentrations. At each passage, viruses that replicated despite the presence of neutralising antibodies were recovered at necropsy. Virus load was quantified using qRT-PCR and whole genome sequencing was performed using the Illumina platform. Additionally, genetic variation within each viral isolate was investigated by whole genome SNP analysis.

There was clear phenotypic and genetic evidence of rapid viral evolution, at both the virus consensus and intra-host level, in response to selection with neutralising antibodies. Of particular note were two amino acid substitutions in the P2 domain of the capsid protein that rapidly became dominant within the virus population, likely because the changes conferred partial resistance to neutralising antibodies. Interestingly, there was also clear evidence for selection of synonymous substitutions, most notably in the viral polymerase and capsid genes. These findings demonstrate proof of concept that RHDV evolution can be experimentally manipulated to select for variants with altered immunological characteristics. This may ultimately lead to the generation of novel RHDV serotypes that are able to overcome pre-existing immunity to currently used rabbit biocontrol strains.

Gentamicin Resistance in MRSA Fight Club: Clone Wars Leading to New Insights

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Methicillin-resistant Staphylococcus aureus (MRSA) continues to be a formidable pathogen in clinical and community settings. Capitalising on a multitude of virulence factors which enable a diverse and complex disease profile, while still ensuring survival though non-pathogenic colonisation, it remains a critical contributor to the burden of infection in Australian hospitals. The heterogeneity of antibiotic resistance profiles between MRSA clones and the seemingly counterintuitive re-emergence of gentamicin susceptible MRSA (GS-MRSA) cannot be accounted for by changing patterns of antimicrobial use alone. Furthermore, the replacement of gentamicin resistant strains (GR-MRSA) by gentamicin susceptible strains (GS-MRSA) suggests clonal resistance.
displacement may be the result of a direct interaction between organisms.

Previous studies have explored MRSA co-culture in broth, demonstrating that one clone ultimately out-competes the other for nutrients and growth factors. This study provides further explanation for MRSA clonal displacement, demonstrating a direct and aggressive inhibition of GR-MRSA by GS-MRSA. Isolates were collected from John Hunter hospital between 2000 and 2014 and selected based on their antibiotic susceptibility profiles. Utilising a variation of agar-overlay culture, GS-MRSA has been shown not only to out-compete GR-MRSA but to directly inhibit its growth through a seemingly bactericidal mode of action. This has been shown to occur not only in large concentrations, but also in cultures where CFUs present are reflective of a clinical burden of nasal colonisation or disease. This phenotypic model was then correlated with sequence data obtained from next generation sequencing with the aim of typing clones and identifying variation at the nucleotide level that may elucidate the mechanism behind bactericidal activity observed in culture. These findings offer novel insight into MRSA clonal interactions and raise questions pertaining to our ideas of fitness in relation to microbial evolution.

Identifying Virulence Switches of Rabbit Haemorrhagic Disease Virus

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Rabbit haemorrhagic disease virus (RHDV) is used in Australia to control the European rabbit, a major agricultural and ecological pest. RHDV causes acute fatal haemorrhagic disease and is closely related to a non-pathogenic virus of rabbits (RCV). Tissue tropism differs between the two viruses, with the benign RCV primarily replicating in the duodenum and the highly virulent RHDV targeting the liver. The genetic similarity of these two viruses with contrasting phenotypes makes them an ideal model system to study the evolution of RHDV virulence, with a particular focus on genes or specific mutations responsible for tissue tropism and virulence.

A total of 29 new RHDV and RCV full length genomes were sequenced using illumina MiSeq technology. Sequences were combined with published sequences (n=128) for evolutionary analysis. As several RHDVs/RCVs recombinants were included in the dataset, phylogenetic analysis was carried out separately for the non-structural protein genes and the capsid protein gene, VP60, which are located either side of the recombination breakpoint.

Virus strains with confirmed liver tropism clustered together in phylogenetic analyses of VP60, although some of these strains are not highly virulent. For example, a moderately pathogenic RCV isolate from Michigan (MRCV) that replicates in the liver clustered with RHDV in the VP60 phylogeny, but with the non-pathogenic RCVs in the phylogenetic analysis of the non-structural protein genes. Nineteen conserved amino acid changes in the non-structural protein sequences distinguish the benign viruses from their pathogenic relatives, nine of these changes occurring in the p16 protein. These findings suggest a key role for VP60 in tissue tropism, with additional genes contributing to the change in virulence. Future investigations into the function of the non-structural proteins, particularly the as yet uncharacterised p16 protein, and their role in virus virulence is clearly warranted.

Isolation of Staphylococcus epidermidis from blood cultures: can virulence factors predict true bacteraemia?

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This study provides further insight into the complexity of C. pecorum epidemiology and may open new opportunities for vaccine development since chlamydial plasmid proteins in other species are immunogenic and potentially important virulence factors [4].
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The clinical significance of the growth of Staphylococcus epidermidis from patient blood cultures is often unclear, presenting uncertainty regarding patient management. In up to 80% of such cases, growth represents a contaminated blood collection, however true bacteraemia requires prompt and directed antimicrobial treatment to prevent poor patient outcomes. Infection with S. epidermidis often results from contaminated indwelling medical devices that include intravascular devices, stents, orthopaedic and cardiac valve prostheses. Clinical presentation of S. epidermidis bacteraemia is typically indolent in comparison to other microorganisms, and patients with indwelling medical devices may be unnecessarily treated with antibiotics and/or replacement of the indwelling device. S. epidermidis' success as an opportunistic pathogen is primarily attributed to its ability to colonise and form biofilms on intravascular devices and evade the host immune system. In this study we analysed 160 strains of S. epidermidis previously isolated from blood cultures of patients from intensive care, oncology or the renal transplant unit. Half of the strains were clinically determined to be causing bacteraemia whereas the remaining half were determined to be contaminants. All strains were interrogated for an array of postulated virulence factors (Vs) to identify those factors that are associated with the bacteraemia. A significant correlation of Vs with invasive strains could be used to develop a multiplex PCR test that would be capable of identifying virulent strains of S. epidermidis and hence assist clinicians with treatment decisions. Ideally, such a test would identify those patients with true bacteraemia, optimising patient outcomes, reducing the costs of health care and resultant infection management issues.

The StkRS two-component system influences colistin resistance in Acinetobacter baylounii

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Multidrug resistant (MDR) bacterial infections are increasing in frequency worldwide, which coupled with a lack of new drugs, places enormous pressure on treatment regimes. Therefore, understanding how bacterial pathogens circumvent treatment is urgently required. Acinetobacter baylounii is a Gram-negative opportunistic hospital-acquired pathogen of which many strains are MDR. As A. baylounii uses a multifactorial process to cause disease these elements are likely to be under stringent genetic control. To provide insight into these regulatory networks we examined the role of a response regulatory element (RR) within the MDR clinical A. baylounii 04117201 isolate. The RR protein is part of a two-component system (TCS), consisting of a membrane-bound histidine kinase and a cognate cytosolic RR, working in concert to respond to environmental changes. The RR stkR of the TCS stkRS was deleted using site-specific recombination. The resulting ΔstkR strain was assessed for alterations in bacterial cell adherence, surface hydrophobicity, and its resistance profile to selected antibiotics including colistin, known as a “last resort” drug. Compared to the parent, the ΔstkR strain demonstrated a 2-fold increase in eukaryotic cell adherence, increased cell surface hydrophobicity and a 2-fold increase in colistin resistance. Protein analysis of the ΔstkR strain grown in the presence of increasing concentrations of colistin identified several proteins differentially expressed compared to the parent strain. Previous studies have shown colistin resistance to be due to major alterations in the lipid A of resistant A. baumannii strains; examination of the surface polysaccharides of the ΔstkR strain revealed only minor changes in this region. Analysis of high throughput RNA sequencing revealed a number of genes that are differentially expressed and has potentially identified the source of the increased colistin resistance, representing a novel mechanism in A. baumannii.

HRV vs. HRV16 – variation in the nuclear effects of Group-A rhinovirus infection

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Human rhinovirus (HRV) is a positive sense RNA virus that is a major cause of upper respiratory tract infections and contributes significantly to virus-induced asthma exacerbations. Despite replicating in the cytoplasm, HRV has a significant impact on nuclear transport and nuclear localisation of host proteins. A number of studies have identified differences between HRV serotypes, with respect to host response, protease activity and replicative ability. Here we report the sero-specific effects of two group A human rhinovirus serotypes, the minor group HRV2 and the major group HRV16, on nuclear transport and nuclear protein localisation. Using Western analysis, immunofluorescence and real time PCR of infected cell samples, we show that HRV2 replicates at a faster rate than HRV16, which correlates with earlier production of viral proteases and disruption of host nuclear transport. In addition, we show the mislocalisation of the host protein SC35 and Sam68 occur at similar time points after infection with HRV2 or HRV16. Interestingly, the mislocalisation of hnRNP-C1/C2 is delayed in HRV16 infection, and appears to correlate with more complete disruption of nuclear pore components. It therefore appears that mislocalisation of select host nuclear proteins occurs irrespective of the extent of nuclear pore degradation.

Microbial Investigation of Diabetic foot ulcer - An Australian Perspective

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Diabetic foot ulcers (DFUs) are one of the most common causes of lower limb amputations, prolonged outpatient care and hospitalization worldwide. Bacterial infection is one of the complications of DFU. As conventional microbiological methods are time consuming and only identifies about 2% of the wound microbiota, detection of bacteria present in DFUs using molecular methods is highly advantageous. The present study was undertaken to determine the bacterial profile of infected ulcers along with the bacterial antibiotic resistance and virulence traits using DNA based techniques. A total of 30 patients with DFUs were included in this study. 223 swab samples were obtained from these patients and DNA was extracted from swabs. The extracted DNA samples were subjected to Polymerase Chain Reaction (PCR) using species-specific primers to detect the three main DFU infecting bacteria, Staphylococcus aureus, Pseudomonas aeruginosa and Enterococcus faecalis. Furthermore, the antibiotic resistance profiles and virulence traits of the
Expression and partial characterisation of rabbit haemorrhagic disease virus non-structural proteins

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Rabbit haemorrhagic disease virus (RHDV) is a highly virulent and species-specific virus of the European rabbit (Oryctolagus cuniculus). Little is known about the molecular biology and virulence mechanisms of RHDV, mainly due to the lack of an effective cell culture system. Many non-structural viral proteins determine critical replication strategies and influence host cell responses to infection. Identifying the intracellular compartment in which viral proteins accumulate is often the key to understanding protein functions and the knowledge of their subcellular localisation may even point to possible host cell interaction partners. To increase our understanding of RHDV replication, we investigated the subcellular localisation of all RHDV non-structural proteins and observed a wide range of different localisation patterns in transiently transfected cells. While some proteins did not associate with any distinct structures or compartments in the cells (e.g. VPg and the protease), others showed a defined subcellular localisation (e.g. p16, p23, the helicase, p29 and the polymerase). Furthermore, we show evidence for oligomerisation of p23 and an ability of the viral protease to cleave the p16:p23 junction in trans, i.e. outside the context of the nascent polyprotein chain. Notably, expression of the viral polymerase alone and in the context of the entire RHDV polyprotein resulted in a redistribution of the Golgi network. This suggests that, similar to other positive-strand RNA viruses, RHDV may recruit membranes of the secretory pathway during replication, and that the viral polymerase may play a critical role during this process.

Keywords: Diabetic Foot Ulcers, Microbiology, Antibiotic resistance, Virulence
Diversity of biofilm formation in uropathogenic Escherichia coli.

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Urinary tract infections are extremely common with around 80% of these are considered the major virulence factors of this species. There are three gingipains two of which, RgpA and RgpB, are specific for cleavage of Arg-X peptide bonds and one Kgp, specific for Lys-X peptide bonds. The gingipains have sequence similarity proposed to derive from their evolving catalytic domain. Biochemical analysis and site directed mutation has indicated that Cys473 of RgpB is involved in catalysis. The crystal structure of RgpB determined in the presence of a small molecule inhibitor confirmed the involvement of this residue in catalysis and revealed His440 is a catalytic dyad partner. However, examination of the structure also indicated the acidic residue Glu381 is essential for catalysis. In Kgp the comparable residue to Glu381 is the acidic residue Asp388. We have recently determined the structure of Kgp in the same enzyme active complex with the small molecule inhibitor. In this structure the acidic residue Glu381 is essential for catalysis. In Kgp the comparable residue to Glu381 is the acidic residue Asp388. The gingipains are currently being investigated as a potential drug target.

COMBINE, an organisation bringing together Australian students and early career researchers in bioinformatics and computational biology

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COMBINE is a student-run Australian organisation for researchers in bioinformatics and computational biology. COMBINE is the official International Society for Computational Biology (ISCB) Regional Student Group (RSG) for Australia. We aim to bring together students and early-career researchers from the computational and life sciences for networking, collaboration, and professional development.
Australia has many research institutes, each with their own cohorts of students. Aside from conferences, there are few opportunities that bring these students together, allowing them to discover the different kinds of research going on at other institutes.

COMBINE aims to bridge this institutional divide by organizing seminars, workshops and social events, and the yearly COMBINE Student Symposium. Together, these events allow students to connect with each other and build a professional network in a casual environment.

Find out more and get involved at http://combine.org.au/  

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The significance of symbiosis in fungi-plant systems is well recognized and represented in the scientific literature. However, fungal-insect symbioses are less well understood, except for a few well known examples, even though they appear to be present in almost every terrestrial ecosystem. Recent molecular investigations suggest that there is a great diversity of fungi associated with insects, potentially forming complex and varied symbioses. These relationships could be of great interest from both an evolutionary and ecological perspective. The close association between the yeast, Kodamaea ohmeri and the small hive beetle, Aethina tumida suggests a symbiotic relationship. A. tumida is a serious pest of European honeybees (Apis mellifera) in Australia and the U.S.A. The yeast K. ohmeri appears to be carried by the adult beetle and has been shown to be associated with the fermentation of hive products during development of A. tumida larvae. In order to explore this relationship, we will establish whether the yeast is an obligate or facultative symbiont of this host, the reciprocity of this relationship, and so determine the contribution of the relationship to beetle bionomics and ecology. This will allow qualitative and quantitative evaluation of the nature of their biological, evolutionary and ecological relationships. These investigations will be used as a model to better understand the role of symbiotic microbes in the evolution of insects and their roles in contributing to better exploitation of certain niches in ecological systems.

Fungal-insect symbiosis: exploring the relationship between the yeast, Kodamaea ohmeri and its host, the small hive beetle, Aethina tumida.

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Fungal infections are very important to the medical community and are a cause of morbidity and mortality, particularly among the immunocompromised. Candida glabrata and Cryptococcus neoformans are two prominent fungal pathogens, causing candidiasis and cryptococcosis, respectively. Although there are now substantial genetic and genomic resources for the study of these organisms, creating and maintaining multiple knockouts in a single organism remains problematic. CRISPR-Cas9 is a novel system for genomic editing and has been adapted to many eukaryotic organisms, recently including the yeasts Saccharomyces cerevisiae and Schizosaccharomyces pombe. Originally discovered as part of the bacterial immune system, DNA segments from invading viruses or plasmids are incorporated into the CRISPR locus. These are transcribed into RNA and direct CRISPR associated (Cas) endonuclease to complementary sequences in the invader’s genome. When Cas introduces a double stranded break, this system has been adapted to create gene disruptions or DNA insertions, as subsequent repair must alter the target site to prevent further Cas cleavage. Guide RNA (gRNA), a ~20 nt sequence specific to the gene of interest, and cloned bacterial Cas protein are introduced into prokaryotic or eukaryotic cells where they allow editing of the target gene. CRISPR-Cas9 has not yet been applied to fungal pathogens, and the aims of this study are to introduce it into the fungus Candida glabrata and Cryptococcus neoformans. Bioinformatic analysis has identified numerous gRNA target sites in multiple fungal pathogens, causing plant diseases and to increase crop yield, has provoked researchers to find out the alternate sources. Therefore, biological control of fungal phytopathogens, and plant growth promotion by use of microorganisms has received an increased emphasis as a safe, environment friendly, long lasting, inexpensive alternative to the chemical fungicides and fertilizers, respectively. In light of this a Streptomyces strain, possessing activity against different fungal phytopathogens viz. Colletotrichum acutatum, Cladosporium herbarum, Alternaria brassicicola, Alternaria mali, Colletotrichum gloeosporioides, Alternaria alternata, Fusarium oxysporum fsp. dianthi and Fusarium moniliforme, was isolated from soil and identified as Streptomyces hydrogenans strain DH16. Application of culture supernatant (5%) and cells (105 cfu/ml) of Streptomyces DH16 as seed and foliar treatments on Raphanus sativus suppressed the disease incidence of black leaf spot caused by A. brassicicola. The metabolites in the extract of S. hydrogenans DH16 showed insecticidal potential with 70 % larval, 66.66 % prepupal and 100% pupal mortality at concentration of 1600 µg/ml. The metabolites also prolonged the larval developmental period along with morphological abnormalities. Additionally, this strain also possesses various plant growth promoting activities viz. indole acetic acid production (1AA; 80 µg/ml), ACC deaminase activity and nitrogen fixation. The in vivo effect of Streptomyces DH16, and IAA produced by it on plant growth promotion was evaluated on pea seedlings (Pisum sativum). Both the treatments showed enhanced seed germination, root length, shoot length, fresh and dry weights, and number of lateral roots. These results demonstrate that Streptomyces hydrogenans DH16 strain as well as its extracellular metabolites (antifungal, insecticidal and plant growth promoting substances) can be exploited as soil amendment as biofungicide, insecticide and biofertilizer.

Genome editing by CRISPR-Cas9 in pathogenic fungi

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Biocontrol and plant growth promoting potential of Streptomyces hydrogenans strain DH16

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Increased public concern about environmental problems caused by the use of agrochemicals, used to control plant diseases and to increase crop yield, has provoked
genes of interest across both organisms, including genes with easily scored phenotypes where we can optimize the system. Successful application of the CRISPR-Cas9 will enable highly targeted editing unhindered by marker availability in these medically important organisms.

Enhancement of Fatty Acids Production from Saccharomyces cerevisiae by Overexpression of Malic enzyme and Heterologous Expression of Thioredoxin from Corynebacterium glutamicum

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Due to an increasing demand of environmentally friendly biofuel, it is necessary to look for the way which continue to provide renewable energy sources. Fatty acids production from renewable feedstock has currently attracted significant attention as an important platform for producing the renewable bio-fuel. Here, we engineered Saccharomyces cerevisiae to enhance fatty acids biosynthesis pathway by insertion of three genes related to fatty acids production. First, the acetyl-CoA carboxylase I (AccI) from Corynebacterium glutamicum was introduced into S. cerevisiae. AccI is most important rate limiting step to produce fatty acids and it converts the acetyl CoA into malonyl CoA. Second, we introduced the putative acyl-CoA thioredoxin (Cgl1664) from C. glutamicum which converts fatty acyl CoA into fatty acids. Third, we overexpressed the malic enzyme I (Mael) to regenerate the NADPH which consumed during the elongation step of fatty acids. The heterologous overexpression of the AccI led to 1.7-fold increase in recombinant yeast YPH499 (pAccI), which represented 220.2 mg/g DCW. Also, YPH499 (pCgl1664) which contains the Cgl1664 showed 232.7 mg/g DCW, which is 1.7-fold higher than wild type. We also overexpressed the Mael in the recombinant strain YPH499 (pMael) and it can produce the fatty acids with 261.5 mg/g DCW, and it was 2.0-fold higher than wild type. Continually, we constructed the shuttle vector paccmaelcgl1664 which has all three genes accI, mael, and cgl1664. This recombinant plasmid was introduced into S. cerevisiae and YPH499 (pAccIMaelCgl1664) can produce the fatty acids of 356.3 mg/g DCW, which is higher than 2.7-fold than wild type. The Mael provide the NADPH to be in balance and the overexpression of AccI and Cgl1664 increase the yield of fatty acids. These results represent the potential to development of an industrial platform effective producing fatty acids and fatty acids-derived molecules.

Cytokine induction by Cryptococcus strains of varying pathogenicity

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Cryptococcosis, a systematic fungal infection caused by pathogenic Cryptococcus species, has emerged as a devastating cause of morbidity and mortality worldwide. It presents with a range of clinical outcomes that result from complex interactions between the pathogen and the mammalian host. During infection, Cryptococcal cells lodge in the alveoli of the lung where alveolar macrophages release cytokines in the response to the presence of antigens. These cytokines modulate the development and expression of T-cells stimulating a particular activation profile. A Th1 response is pro-inflammatory and leads to pathogen killing and clearance while a Th2 response is anti-inflammatory and leads to fungal growth.

In this study we are examining cytokine induction using two sets of Cryptococcus strains: a collection of 72 C. neoformans and C. gattii clinical isolates from two hospitals in Botswana, and a set of seven strains that have been derived from the virulent type strain C. neoformans H99 that vary in virulence despite being almost identical at the genotypic level. To assess host immune responses induced by different strains, J74.1 macrophages were infected with each isolate, the cells lysed, and purified RNA obtained. Cytokine expression was assessed by RNase Protection Assay (RPA) where radiolabelled anti-sense RNA probes are hybridised to purified macrophage RNA; non-hybridising RNA is degraded, and the protected RNA is electrophoresed, resulting in a profile of bands corresponding to the different cytokines.

The results to date have revealed substantial variation in the amounts of TNF-α, IL-1β, and IL-1α induced by the different clinical isolates. However, statistical analysis found no significant association between cytokine profile and genotype or clinical outcome. We are currently optimising this analysis to ensure that it is thoroughly standardised and reproducible across all infection assays, and extending it to the H99 derivative strains.

Dissecting the response to fluconazole in susceptible and resistant strains of Cryptococcus gattii

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Cryptococcus gattii is a fungal pathogen capable of causing respiratory and systemic infection with potentially fatal sequelae. Infections are treated with antifungal induction therapy using amphotericin B and fluconazole, followed by maintenance therapy with fluconazole. Fluconazole prevents the biosynthesis of ergosterol and causes toxic sterol intermediates to accumulate, resulting in stress to the cell membrane. Strains of C. gattii exhibiting intrinsic resistance to fluconazole are emerging. Chong et al (PLOS ONE, 2010) used proteomic analysis to study the growth dynamics and protein expression profiles of intrinsically resistant strains of C. gattii in response to fluconazole and compared these with strains exhibiting typical fluconazole susceptibility. Differentially expressed proteins were represented as features in a protein interaction network. The aim of the current study is to further analyse and validate significant proteins in these networks. Using the latest gene annotation data and a new network analysis program, genes with a role in coordinating an effective cellular response to fluconazole, particularly in the resistant strain, were identified. These included a satellite network grouping ATP synthase subunits with high level of interconnectivity that had strain-dependent regulatory
responses: HCR1, a highly connected hub protein that was highly down-regulated in the resistant strain. In addition, ontological sub-grouping of the network highlighted GRX5, which plays a role in oxidative stress response and was significantly up-regulated in the resistant strain. Deletion of these genes in Saccharomyces cerevisiae resulted in decreased resistance to fluconazole and various other stressors. Identifying how these cellular processes and pathways interact to coordinate an effective fluconazole response may allow new targets for novel antifungal treatments to be discovered.

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**In-vitro antibacterial activity of underutilized plant crude extracts against food-borne pathogens**

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There is emerging research interest to discover new antimicrobials from plant sources for food preservation and control of infectious diseases. The antibacterial activity of underutilized plants “Rankihiriya” (Alpinia malaccensis), Purpurata (Alpinia purpurata), “Harankaha” (Cucurna albiflora Thwaites) and “Kottamba” (Terminalia catappa) were evaluated against twelve food-borne pathogens. Crude hexane extracts of all plant rhizomes were obtained, and T. catappa ethanol extract was obtained from the red peri-carp of fruit. The antibacterial activity was determined using agar disc diffusion assay and broth dilution assay. Gas Chromatograph-Mass Spectrometry analysis was performed to the plant with the strongest antibacterial activity to determine chemical composition. Among the four plant extracts, A. malaccensis extract had significantly (p<0.05) higher diameter of inhibition zone ranging (33±1.29 to 40±0.52 mm) against six Staphylococcus aureus strains including methicillin resistant S. aureus compared to Listeria monocytogens. Escherichia coli and Salmonella Typhimurium Also, T. catappa showed higher diameter of inhibition zone (19±1.15-20±0.57 mm) against S. aureus strains except S. aureus MSSA SS 21D and other bacteria tested. However, C. albiflora showed the significantly (p<0.05) lowest diameter of inhibition zone for all bacteria strains tested. The minimum inhibitory concentration of the A. malaccensis extract was 0.625 mg/ml, and the minimum bactericidal concentration was 5 mg/ml for all S. aureus strains tested. Gas Chromatograph-Mass Spectrometry of A. malaccensis extract showed a major unknown compound of 82.87% by Willy Library W9N08 while other compounds were, 1,4 dihydrophenanthrene (6.05%), Trans beta faresene (2.64%) and Farnesol, acetate (2.32%). Even though, the major chemical compound was unknown, its mass spectrum was similar to the mass spectrum of Alpinia galanga. Therefore, we speculate that the, major chemical compound could be 1’1’-acetoxy-chavicol acetate and need to further confirm by Nuclear Magnetic Resonance. This study confirmed that uncharacterized, underutilized crops have potential antibacterial activity against food-borne bacteria.

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**Antibacterial and antibiofilm activity of Alpinia galanga (Galangal) against gram positive bacteria and evaluation of pharmacological safety.**

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The bioactive compounds discovered from different plant species have become a gold mine in future therapeutics. The current study focus on authentification of the correct plant species of_A. galanga grown in Sri Lanka, determination of antimicrobial, antibiofilm activity, analysis of chemical composition and evaluation of pharmacological safety of the crude rhizome extract. The antibiofilm activity of the crude galangal hexane extract was tested against six strains of Staphylococcus aureus and four strains of Listeria monocytogens using disk diffusion assay and broth dilution assay. 96 well microtitre plate assay was used to test antibiofilm activity against S. aureus 113. The chemical composition of the crude rhizome extract was investigated using Gas Chromatographic and Mass Spectrometric analysis. Pharmacological safety was evaluated using a standard method for acute oral toxicity. All strains of S. aureus tested showed significantly (p<0.05) higher diameter of inhibition zone ranging from 36 mm to 44 mm including methicillin resistant strain compared to L. monocytogens. The Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of the extract for the six strains tested were found to be 1.25mg/ml and 5mg/ml respectively at 24 hr and did not change after 48 hr except S. aureus MSSA 21D. Crude extract 20 mg/ml showed a significant (p<0.05) lower absorbance (0.299 ± 0.05 nm) at 595 nm in biofilm assay which is 80.68% reduction in biofilm adherence compared to the control. The major chemical compound 1’acetoxychavicol acetate (82.88%) was found to be in highest concentration reported so far. According to the acute oral toxicity study 2000 mg/kg body weight of extract was not toxic to Wistar rats. In conclusion, A. galanga crude extract may be effective as an alternative antibiotic agent to combat antibiotic resistant S. aureus in pharmaceutical industry and as a natural preservative and a disinfectant in food industry to control S. aureus.

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**Screening of a bacteriocin-producing Bifidobacterium with antibacterial activity against Clostridium difficile**

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Bacteriocins are secreted proteins produced by lactic acid bacteria (LAB) in early stationary phase of growth, which can inhibit the growth of similar or closely related bacteria. In this study, we investigated the antibacterial activity of a bacteriocin-producing strain of Bifidobacterium against Clostridium difficile. The bacteriocin was purified and characterized using standard techniques. The bacteriocin had a molecular weight of approximately 50 kDa and was active against C. difficile ATCC 43300. The minimum inhibitory concentration (MIC) of the bacteriocin against C. difficile was determined using the broth microdilution method and was found to be 6.25 μg/ml. The bacteriocin was found to be heat-stable and resistant to proteolytic enzymes. This study highlights the potential of bacteriocins as an alternative to antibiotics for the treatment of Clostridium difficile infection.
study, we attempted to screen *Bifidobacterium* isolate that produced bacteriocin exhibiting antibacterial activity against *Clostridium difficile*. Among 800 LAB strains isolated from calf fecal samples, 620 strains (77.5%) exhibited antimicrobial activity against *C. difficile*, of which 21/620 were identified as *Bifidobacterium* sp. Neutralized cell-free supernatant (NCFs) of the selected *Bifidobacterium* isolate, BD-1, showed an antimicrobial effect against not only drug resistant *C. difficile* strain 630 but also *Bacillus subtilis*, *Pediococcus pentosaceus* and *E. coli* ETEC. The maximum growth observed by optical density was reached approximately 5.8 after 18 h of incubation. Furthermore, the production of antimicrobial compound was found at the late exponential phase of growth (12h) and reached a maximum activity during the stationary phase. The anti-bacterial property of the NCFs fraction of the BD-1 strain was heat (100°C for 30 min) and pH-stable (pH 3-9); however, it was sensitive to protease treatment. Our findings suggested that the substance(s) in NCFs of *Bifidobacterium* isolate BD-1 could potentially be a bacteriocin and may be a good candidate for alternative antimicrobial agents to treat *C. difficile* infection.

### Potential use of *Pediococcus* sp. isolated from Thai fermented sausage as probiotics

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Over the past decades, antimicrobial activity of lactic acid bacteria (LAB) has been studied and used as probiotics, which have protective effect against pathogenic bacteria including *Clostridium difficile*. *C. difficile* is an infectious pathogen that can cause gastrointestinal disorders ranging from mild diarrhea to pseudomembranous colitis. There are concerns on antibiotic uses to eradicate *C. difficile*: alternative strategies to treat and prevent *C. difficile* infection are therefore of interest. In this study, the probiotic potential properties of *Pediococcus pentosaceus*, LAB isolated from Thai fermented sausage called nham, were evaluated and the inhibitory effect against *C. difficile* was also studied. Neutralized cell-free supernatant of *P. pentosaceus* showed the inhibitory effect against pathogenic bacteria including *C. difficile*. The survival in simulated gastric and intestinal fluids as well as adhesion ability to colonic cells were studied. The results showed that *P. pentosacueus* could survive under tested gastric fluid at pH 3 for 3 h and under basic (pH 8) condition of intestinal fluid for the following 3 h. This strain was also able to adhere to colonic cells with logCFU/ml of 3.94 (66%). Our findings suggest that *P. pentosaceus* isolated from nham would be a good candidate as probiotics against *C. difficile*.

### Heterologous expression of an ABC transporter from *Clostridium difficile*

**Chawalit Ngemsomboon**, 1 Tavan Janvilioti 1, 2

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*Clostridium difficile* is an important nosocomial pathogen that causes infectious diarrhea and pseudomembranous colitis among patients in hospitals worldwide and also poses a serious problem in farming animals. Recently, the incidence and severity of *C. difficile* infection has markedly increased due to resistance to many antibiotics. One of the mechanisms that have been associated with antibiotic resistance in *C. difficile* is through ATP binding-cassette (ABC) transporters which are well implicated in bacterial virulence and pathogenicity, making them potential targets for the development of new antimicrobial therapeutic strategies. The genome of *C. difficile* 630 was screened for sequences encoding putative ABC transporter proteins homologous to multidrug resistance ABC transporter in *C. hathewayi*. The putative ABC transporter gene was cloned into the pET28A expression vector and functionally expressed in *Escherichia coli*. The corresponding protein was detected and function of this protein was analyzed in antibiotic susceptibility assay. ABC transporter-expressing cells showed higher half minimal inhibitory concentration of antibiotics compared to the control cells. Therefore, ABC transporter might function as a multidrug transporter involved in multiple antibiotic resistance.

### Antimicrobial and chlorhexidine mouthwash resistance of dental plaque bacteria

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Chlorhexidine is mainly accepted as disinfectant in hospital settings and is also used for oral hygiene in oral care formulations as in mouthwashes/mouthrinses. In Pakistan self medication with antimicrobial agents is common and increasing bacterial antimicrobial resistance is occurring, thus adequate disinfection procedures are important. In this study bacteria isolated from dental plaque were selected at random for antimicrobial resistance analysis. These bacteria were characterized by 16S rRNA sequencing as *Chryseobacterium culcis*, *Chryseobacterium indologenes*, *Acinetobacter johnsonii*, *Enterobacter ludwigii*, *Streptococcus salivarius*, and *Pseudomonas stutzeri*. Antibiotic resistance profiles were measured using the disc diffusion method. *Chryseobacterium* species were found to be more resistant to multiple drugs including ampicillin, kanamycin, gentamycin, and tetracycline. *S. salivarius* and *P. stutzeri* had high and intermediate susceptibility to ampicillin respectively whilst the other species were resistant to this drug. These dental bacteria showed diversity in biofilm forming ability with *P. stutzeri* found to be the most biofilm producing, *P. stutzeri*, *A. johnsonii*, and *E. ludwigii* growth was inhibited with chlorhexidine gluconate (Sigma) at 3.8 μg/ml in comparison with *Chryseobacterium* species, with minimum inhibitory concentration of 32 μg/ml. The efficacy of
chlorhexidine available from three different sources to inhibit growth of these species was assessed. Antiseptic solution marketed to dental clinicians (Dentalfie) was found more effective against bacterial growth in comparison with other products. Bacterial biofilm grown in a 7% well microtitre plate were exposed for different time intervals to a commercial chlorhexidine-based mouthwash (chlorhexidine gluconate 0.2%). The bacteria, particularly the Chryseobacterium sp. could survive up to 30 second exposures. Our results indicate that antimicrobial resistant bacteria were readily isolated from dental plaque and that chlorhexidine-based mouthwash should be used to mouth rinse for at least 40 seconds to inhibit the growth of chlorhexidine resistant dental plaque species.

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A preliminary examination of the anti-microbial properties of methanolic fractions obtained from three species of Russula

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The development of antimicrobial resistance is of world-wide concern, and has the potential to lead to an era where the causative agents of once treatable infections return to their pre-antimicrobial state. This will ultimately result in greater health care costs and increasing death rates.

Fungi have already proven to be a potentially valuable source of novel bioactive compounds, including those demonstrating anti-microbial, anti-oxidant and/or anti-cancer properties. However to date, the metabolites of only a small number of fungal species have been therapeutically examined.

Russula spp are members of a basidiomycetous fungal family, commonly found in Australian temperate forests. While not known for their anti-microbial properties, research in this area has also been spasmodic and inconsistent, employing a wide variety of extraction methods and testing protocols. It has also included very few Australian species. Through the taxonomic investigation of these species in this PhD project, the opportunity has arisen to also examine some of their metabolic properties.

Methanolic extracts were prepared from three species of Russula. Following drying and pre-adsorption onto C18-bonded silica, seven duplicate fractions were collected via preparative HPLC. These were subsequently dried and resuspended at a concentration of 20mg/ml in 100% DMSO. Pathogenic microbes including E. coli, Staphylococcus aureus and Candida albicans were exposed to the preparations following a modified well diffusion technique.

This presentation will explore the results obtained, difficulties experienced, and future directions of this aspect of this project.

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Antimicrobial effect of hypertonic saline on Pseudomonas aeruginosa isolates from cystic fibrosis lung infections

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Introduction

The development of antimicrobial resistance is of world-wide concern, and has the potential to lead to an era where the causative agents of once treatable infections return to their pre-antimicrobial state. This will ultimately result in greater health care costs and increasing death rates.

Fungi have already proven to be a potentially valuable source of novel bioactive compounds, including those demonstrating anti-microbial, anti-oxidant and/or anti-cancer properties. However to date, the metabolites of only a small number of fungal species have been therapeutically examined.

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Detection of Carbapenemase-producing Enterobacteriaceae in KK Women’s and Children’s Hospital, Singapore.

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Carbapenemase-producing Enterobacteriaceae (CPE) are an emerging problem in hospitals. Patients transferred between healthcare facilities may require isolation to rule out multidrug resistant organisms. This can put a strain on resources due to the limited number of isolation beds.
available. Thus, there is a need to have a rapid, inexpensive and reliable diagnostic test to detect the presence of CPEs. We had evaluated a recently published test (CarbaNP) developed by Nordmann et al. (2012) and introduced it to identify CPE from patient samples in our hospital. Rectal swabs or stool from patients were streaked onto ChromID Carba medium (Biomerieux) and incubated at 35°C overnight. Suspected pink and blush-green colonies from the ChromID Carba medium were isolated on sheep blood agar and sensitivity test was done for meropenem. Meropenem resistant colonies were subsequently tested using CarbaNP test, Modified Hodge Test and Rosco KPC/MBL Confirm Kit (Rosco Diagnostica, Denmark) with Temocillin tablet. Polymerase-Chain reaction (PCR) was performed on CarbaNP positive samples to confirm the presence of New Delhi metallo-beta-lactamase (NDM), Klebsiella pneumoniae carbapenemase (KPC) and OXA48-like carbapenemase. One percent (4/445) of the samples collected in 2014 were positive for the CarbaNP test. They were all NDM positive by PCR method. None of the strains were found to contain other carbapenemase genes such as OXA48 or KPC. Although there were few positives, we found the introduction of the CarbaNP test to be useful as we could rule out the presence of CPE a day earlier than the Modified Hodge Test or Rosco KPC/MBL Confirm Kit. This will help to minimize the spread of CPE and improve patient management.

Sub-inhibitory doses of aminoglycoside antibiotics induce changes in the phenotype of Mycobacterium abscessus

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Sub-inhibitory doses of antibiotics have been shown to cause changes in bacterial cell morphology, biofilm formation, and resistance to antibiotics. In this study, the effects of sub-inhibitory doses of aminoglycoside antibiotics on M. abscessus were investigated. Treatment of M. abscessus cells with sub-inhibitory doses of amikacin was found to change their colony morphology from a smooth to a rough morphology and increase their ability to form biofilms, aggregate in culture, and resist phagocytosis and killing by macrophages. M. abscessus cells treated with a sub-inhibitory dose of amikacin also became more potent in TLR-2 stimulation, leading to increased TNF-a production by macrophages and severity of lung inflammation. The mab_3508c gene was shown to play a major role in mediating these phenotypic changes as its expression in M. abscessus cells was increased when they were treated with a sub-inhibitory dose of amikacin. In addition, over expression of mab_3508c in M. abscessus cells caused changes similar to those induced by sub-inhibitory doses of amikacin, including smooth to rough switching in colony morphology, increased ability to aggregate in liquid culture, decreased motility, and increased resistance to killing by macrophages.

Deciphering the molecular mechanisms of ertapenem resistance in Klebsiella pneumoniae

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Introduction:
Thioguanine (TG), a guanine antimetabolite, is a thiourine drug used to treat some inflammatory conditions. TG is metabolised to thioguanine nucleotides (TGNs) via the hypoxanthine guanine phosphoribosyl transferase enzyme of the purine salvage pathway. TGNs exert immunosuppressive actions by antagonising RAC1 in lymphocytes. Interestingly, TG treatment was also associated with distinct population shifts in the caecal mucosal microbiome of C57Bl/6 treated mice. We envision that the shift in microbiome might be due to metabolism of TG via purine salvage pathways.

Hypothesis:
The shift in the mucosal microbiome may be due to bacterial metabolism of TG to TGNs and that TGNs production may be associated with an antibiotic effect.

Aim:
The aim was to determine TGNs production by a range of colon bacilli when incubated with TG. And also to correlate the TGNs production with the bacterial growth to determine the antibiotic effect.

Methods:
The strains tested so far include Escherichia coli strains (DH5α, PC1101 and BL21) and Enterococcus faecalis. The E. coli strains and E. faecalis were grown in LB and BHI broth respectively. Bacterial growth in the presence of TG was determined by optical density (OD600). The production of TGNs was measured using a novel in-vitro HPLC-UV assay. A 1:50 dilution was selected for all of the strains tested apart from E. coli BL21 where 1:100 was selected based on growth curve.

**Results:**
All the tested strains metabolised TG into TGNs. Interestingly, only E. coli DH5α growth showed a dose response inhibition with TG; the other strains were resistant to TG in these conditions. The bacterial growth did not appear to correlate with TGNs production.

**Conclusion:**
TGNs may have selective antibiotic effect which may still be sufficient to alter the caecal mucosal microbiome.

**Significance:**
TG might have an antibiotic effect in addition to immune suppressive actions. Future work will involve further surveying the TG effects on broad range of caecal mucosal microbiome.

**Investigating the Mechanism of Action of the Novel Antimicrobial BDM-I**

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Vancocycin-resistant Enterococci (VRE) and methicillin-resistant Staphylococcus aureus (MRSA) represent two of the most common causes of hospital-acquired infections worldwide, with the latter also associated with community-acquired infections. Both VRE and MRSA comprise part of the “ESKAPE” group of pathogens due (in part) to their ability to readily acquire resistance to multiple antibiotic classes. Due to the increasing problem of antimicrobial resistance, and lack of drug development, it is essential that new/novel antimicrobial compounds are developed.

**BDM-I** represents a novel anti-infective compound being developed by the Australian biotechnology company BioDiem that has a broad spectrum of activity. Importantly, this compound displays activity against multi-drug resistant bacteria such as VRE and MRSA, indicating its potential as a treatment option for related infections. Currently, the mechanism of action of BDM-I remains unknown, however previous studies have indicated that it possibly targets protein phosphorylation.

Following the long-term exposure of VRE clinical isolates to BDM-I, we have been able to generate mutants with elevated minimum inhibitory concentrations (MICs). Utilising these mutants, whole genome sequencing and proteomic analysis was performed in order to identify mutations and changes in protein expression that may be associated with observed MIC increases. Preliminary data analysis has identified mutations within the ATP synthase operon of all sequenced mutants; relatable results were also observed following proteomic analysis.

**Analysis of the compensatory mechanisms for the loss of major porins in Klebsiella pneumoniae**

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The aim of this study is to investigate the mechanisms of compensation for the lack of major porins in Klebsiella pneumoniae under different environmental conditions. Porins form water-filled channels that allow the passive transport of small molecules in and out the bacteria, a vital process to cell survival. Because of their localization in the cell, porins must play a crucial role in the adaptation and interaction between the environment and the bacteria; and also in maintaining the integrity of the cells. It has been suggested that the loss of certain porins could significantly affect the bacterial fitness and the susceptibility to antibiotics. In K. pneumoniae there are two major porins, OmpK35 and OmpK36. It has been suggested that other porins such as OmpK37 or PhoE can compensate the loss of OmpK35/36, playing an essential role for the microorganisms in the absence of these major porins. However, the mechanisms regulating this process have been poorly investigated. In order to fully understand the role of porins in bacterial adaptation and antibiotic resistance, we have constructed simple and double mutants in all the porins of K. pneumoniae wild-type strain ATCC 13883. The level of expression of porins were measured in K. pneumoniae mutants by real time RT-PCR under different conditions (low and high osmolarity) and distinct growth phases (log, logarithmic and stationary phase) and compared with those in the wild type strain. Our data reveal that the adaptation of K. pneumoniae to external conditions in the absence of OmpK35/36 is a complex process that includes the up-regulation and down-regulation of “secondary” porins (ompK37, ompK26, phoE and lamB) and the levels of expression of these genes vary depending on the environmental circumstances and bacterial growth phase.

**Antimicrobial resistance and resistance genes in recent Enterobacter isolates from NSW**

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Emerging antimicrobial resistance is a global concern, with multi-resistant organisms causing difficulties in the management and treatment of severe infections. Enterobacter species are among the most commonly isolated Enterobacteriaceae implicated in clinical infections. We investigated the prevalence of antibiotic resistance in 1466 Enterobacter isolates (mainly from urine and blood) from the Centre for Infectious Diseases and Microbiology, Westmead, January 2012–April 2015. Minimum inhibitory concentrations were determined using the Phoenix™ Automated Microbiology System and results interpreted using Clinical and Laboratory Standards Institute guidelines. A subset (n=550) of these Enterobacter isolates were screened for genes commonly responsible for reduced susceptibility to β-lactams (extended-spectrum β-lactamase.
In-vitro adhesion to buccal epithelial cells, denture acrylic surfaces and relative cell surface hydrophobicity of oral Candida species following limited exposure to sub-therapeutic concentrations of chlorhexidine gluconate

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Candida albicans as well as its non-albicans counterparts such as Candida tropicalis, Candida krusei, Candida glabrata and Candida dubliniensis are aetiological agents of oral candidosis. Their adherence to buccal epithelial cells (BEC), denture acrylic surfaces (DAS) and cell surface hydrophobicity (CSH) are attributes associated with yeast colonization and infection. Chlorhexidine gluconate (CG) is a widely used antiseptic in dentistry due to its excellent antibacterial properties, but differences in the proportions of genes found suggest subtly different gene pools.

Macrolide therapy for chronic lung disease: significant implications for oropharyngeal dysbiosis and the selection of resistance

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Macrolide antibiotics are used increasingly to prevent recurrent exacerbations in patients with chronic lung diseases due to their clinical efficacy across a range of respiratory conditions. However, the impact of their long-term use on wider commensal systems, such as those within the oropharynx, is not known. There are serious concerns regarding the implications of prolonged antibiotic exposure on both the disruption of commensal microbiota, and the potential transmission of antibiotic resistant pathogens. To investigate the impact of macrolide use on commensal microbiota, culture independent analysis was performed on oropharyngeal swabs collected from patients with non-cystic fibrosis bronchiectasis, as part of a randomised controlled trial of low-dose erythromycin therapy. Paired-end 16S rRNA gene sequencing, targeting the V1-V3 region, was used to characterise oropharyngeal microbiota composition in 30 patients following four weeks of erythromycin or placebo treatment. Changes in macrolide resistance gene carriage (ermA, ermB, ermB, mefA and msrA) were firstly determined using a robust multiplex PCR screen, and then by quantitative PCR normalised to the total bacterial load.

Patients who received erythromycin had significantly reduced oropharyngeal microbiota evenness (Simpson index, p<0.001, t-test) and diversity (Shannon index, p<0.001, t-test), with the most substantial contributions to compositional change made by taxa within the Firmicutes, Bacteroidetes and Fusobacteria phyla. Quantitative PCR analysis indicated that erythromycin exposure resulted in a significant increase in macrolide resistance gene carriage (p<0.05, Wilcoxon test), with changes seen in several resistance determinants.

Despite the low dose and the relatively short period of treatment, these data revealed that macrolide therapy has profound disruptive effects on the oropharyngeal microbiota and strongly selects for resistance carriage. These findings highlight the potential for systemic antibiotics to
trigger dysbiosis in commensal bacterial populations, a potentially important phenomenon that received relatively little clinical consideration.

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The genetic contexts of \textit{blaCMY-2} give hints to its transmission in \textit{Escherichia coli}

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\textit{blaCMY-2} is the most prevalent plasmid-borne AmpC β-lactamase gene in \textit{Escherichia coli} globally and confers resistance to cephalosporins and β-lactamase inhibitors. \textit{blaCMY-2} was captured from the chromosomes of \textit{Citrobacter freundii} by IS\textit{Ecp}1 and is invariably found with this insertion sequence in a variety of different genetic contexts and plasmid lineages. \textit{IncI} plasmids appear to be important in the transmission of \textit{blaCMY-2} among \textit{E. coli} populations, including in Australia. We previously sequenced an \textit{IncI} plasmid from Sydney and identified a novel \textit{CMY-2} context in which IS\textit{Ecp}1 is truncated by IS1294, leaving only 161 bp. A 159-bp fragment of \textit{IncA/C} plasmid backbone downstream of \textit{blaCMY-2} suggests mobilisation from an \textit{IncA/C} plasmid by IS1294. A 2.3 kb region adjacent to the right end of IS1294 was missing compared to other \textit{IncI} plasmids. PCR identified plasmids with similar insertions in over 40 diverse \textit{E. coli} isolates in Sydney over a number of years. Sequencing of a subset of these isolates with Illumina MiSeq technology identified related \textit{IncI} plasmids that do not have a deletion adjacent to IS1294, as well as examples of four different sized deletions. There were few differences across the backbones, except in regions related to entry exclusion and conjugal transfer that may represent functional differences. The unique \textit{blaCMY-2} insertion and the similarities between these plasmids suggest that they originated from a common ancestor and subsequent diversification may have occurred via deletions and homologous recombination. Two almost identical plasmids (<10 SNPs) isolated from the same \textit{E. coli} sequence type five years apart may indicate clonal spread of a \textit{blaCMY-2}-containing \textit{E. coli} strain. The dissemination of \textit{blaCMY-2} in Sydney thus appears to be multifactorial, involving clonal spread of \textit{E. coli} strains and horizontal transfer of related, but not identical, \textit{IncI} plasmids to diverse \textit{E. coli} subtypes.

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Type Ib Partitioning Systems of Gram-positive Hospital Pathogens

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Strains of methicillin-resistant \textit{Staphylococcus aureus} (MRSA), coagulase-negative \textit{staphylococci} (CNS), and vancomycin-resistant enterococci (VRE) that are resistant to multiple antimicrobial agents, are a major cause of hospital-acquired infections around the world and are responsible for significant morbidity and mortality. These Gram-positive pathogens commonly contain multiresistance plasmids that encode partitioning systems, which increase their segregational stability. Such systems therefore contribute to the prevalence and spread of these plasmids, and effectively maintain resistance even in the absence of selection. Three types (I-Ill) of partitioning systems are recognised based on centromere structure and type of motor/DNA binding protein.

We have identified putative Type Ib partitioning systems on resistance plasmids from clinical \textit{staphylococcal} and \textit{enterococcal} isolates, which contain candidate centromere-like site(s), and \textit{parA} and \textit{parB} genes that are predicted to encode a deviant Walker ATPase motor protein and a ribbon-helix-helix DNA binding protein, respectively. Subsequently, we have shown that these partitioning systems, when cloned into mini-replicons, confer enhanced segregational stability in their respective hosts. Furthermore, in context of the \textit{staphylococcal} system, we have shown that the putative \textit{ParB} protein regulates \textit{par} operon transcription and that a \textit{ParA-GFP} fusion protein, which is functional in the absence of wild-type \textit{ParA}, localises to the nucleoid; we are also currently examining the cellular localisation of other \textit{par} system components.

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Characterisation of the mobile genetic elements in the \textit{IncPa} plasmids R702 and R938

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Broad host range \textit{IncP} group plasmids are important contributors to horizontal gene transfer and the spread of antimicrobial resistance. They are comprised of six main lineages (a, b, g, e, d and h) based on phylogenetic differences in \textit{trfA}. They share a similar “backbone” of ~40 kb suggesting that all have evolved from a common ancestor. The backbone contains relatively few restriction endonuclease cleavage sites although pronounced clustering of such sites reflects the insertion of foreign DNA (“genetic load”). The regions between \textit{Tra1-Tra2} and \textit{oriV-trfA} are the main foci for attraction of genetic loads in the best characterised plasmids (\textit{IncPb}). The diversity observed among the \textit{IncPb} plasmids is poorly characterised among the other \textit{IncP} plasmids.

R702 and R938 are \textit{IncPa} plasmids isolated from clinical sources in the 1970s. R702 (\textit{Km}, \textit{Tc}, \textit{Sm}, \textit{Su} and \textit{Hg}) and R938 (\textit{Km}, \textit{Tc}, \textit{Sm}, \textit{Su}, \textit{Hg}, \textit{Ap} and \textit{Cm}) encode a range of antimicrobial resistance genes. These genes are thought to be associated with transposable elements embedded within the plasmids. Each plasmid contains a genetic load within the \textit{oriV-trfA} region but not in the \textit{Tra1-Tra2} region. Both R702 and R938 plasmids contain an additional genetic element in an unusual site within the plasmid backbone i.e., R702 in \textit{kldE} and R938 adjacent to \textit{kfrA}. The four genetic elements appear to be intact transposable elements that belong to the \textit{Tra3}-family.

R702 carries a novel cryptic transposon within the \textit{oriV-trfA} region with a unique module of genes encoding proteins of unknown functions. A \textit{Tra2}-like element is present in the \textit{kldE} gene of R702 and in the \textit{oriV-trfA} region of R938. The other element in R938 contains a nested transposon that appears to have evolved by multiple insertion and deletion events. These findings reveal novel attributes of the structure and evolution of \textit{IncPa} plasmids and their transposable elements.
Secretion of proteases by Scedosporium aurantiacum, an emerging fungal pathogen

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Scedosporium aurantiacum is an opportunistic filamentous fungus frequently isolated from the sputum of cystic fibrosis patients in Australia. At the moment, very little is known about the infection mechanism of S. aurantiacum. Secreted proteases have been shown to contribute to fungal virulence, as demonstrated in the studies with e.g. Aspergillus fumigatus and Candida albicans, in which secreted proteases have been found to cause tissue damage or compromise proteins involved in immune response. Here we compared the profiles of secreted proteases between a high-virulence cystic fibrosis clinical isolate WM 06.482 and a low-virulence environmental strain WM 10.136, in shake flask cultivations. The results showed that proteases secreted by the clinical strain consisted predominantly (over 80%) of elastase-like and trypsin-like serine proteases. Aspartic proteases were responsible for 10% of the total protease activity and other aminopeptidases contributed the last 10%. Overall, the activity of the elastase-like, trypsin-like and aspartic proteases was from 5 to 50 fold higher (depending on the protease type) in the clinical strain compared to the environmental strain. Therefore, it suggests that these classes of proteases may contribute to the virulence of Scedosporium. Protease activity of the two strains was further studied by zymogram gel assays. The results indicated two protease bands, with molecular weights of approximately 26 kDa and 60 kDa respectively, were produced by the clinical strain only. The corresponding proteins were identified as a C78 family peptidase and an aspartyl aminopeptidase by mass spectrometry, searched against all existing fungal database in GPM. Upon availability of the Scedosporium genome sequence, we plan to isolate genes responsible for the synthesis of proteases, specifically those produced by the clinical strain.

Analyses of the global secretome and whole cell proteome of a recent Bordetella pertussis epidemic strain

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Background: Bordetella pertussis is one of the most prevalent vaccine preventable diseases in Australia. The number of cases of B. pertussis has increased in the past decade even with high vaccination rates. Several factors have been hypothesised for the re-emergence of B. pertussis including waning immunity and pathogen adaptation. Despite decades of vaccination, our understanding of the biology of B. pertussis remains limited. We aimed to characterise the global secretome and whole cell proteome of L1423; a clinical isolate from the most recent Australian epidemic in 2008-2012. Methods: L1423 was grown in THUJ media at 37°C under shaking conditions for 12 hr. The supernatant was separated from the whole cell with centrifugation and then purified with a 3kDa filter. The whole cells were lysed with sonication and proteins were extracted and purified using acetone precipitation. We then used LC-MS/MS to characterise the proteins found in the whole cell extract and supernatant. Results: In the secretome, 140 proteins were identified with 74 proteins predicted to be secreted bioinformatically. Functional categories were assigned and the majority of proteins found were related to the cell surface with 31 proteins. Twenty three proteins were also associated with pathogenicity in the supernatant. Nine hundred and forty three proteins were found in the whole cell extract. The majority of these proteins were associated with cell surface and macromolecule synthesis or modification. Forty four proteins were also found to be associated with virulence. Conclusion: This is the first study to characterise the global secretome and whole cell proteome of an Australian epidemic B. pertussis isolate.

Comparative analysis of Methicillin Resistant Staphylococcus aureus (MRSA) using a novel proteomic approach

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Staphylococcus aureus is a prominent bacterial pathogen responsible for an array of human diseases in both hospital and community settings, which underpins a huge financial burden worldwide. Emergence of methicillin resistant Staphylococcus aureus (MRSA) initially in hospitals (HA MRSA) and later in community (CA MRSA) has added more challenges both therapeutically and financially. Knowledge of the pathogenesis and virulence factors of this bacterial pathogen remains incomplete despite intense research and alarming, the distinction between HA MRSA and CA MRSA is becoming blurred due to replacement of hospital clones by dominant and more virulent CA MRSA. We investigated the differential proteomics of four clinical MRSA strains (two HA MRSA and two CA MRSA) in three biological replicates, in vitro at stationary (OD600=1) and log (OD600=0.4) growth phases using a novel proteomic approach. Proteins were extracted using an optimized extraction protocol coupled with FASP digestion. Mass spectrometric data acquisition was performed in both data-independent and data-dependent methods using a TripleTOF 6600+. Using the raw data from data-dependent acquisition, a protein database was constructed using the Paragon algorithm to acquire peptide-spectrum matches. This database was then used to extract quantitative information from raw data-independent mass spectrometry files. Multi-strain protein clustering by sequence identity was determined. Quantitative and relative information was assigned to protein clusters, normalised and analysed for significant abundance differences. Protein clusters with differential abundance is used to interpret the functional
characteristics of the significant proteins of interest. This study has revealed unique protein profiles for each of the clinical strains and provides important information for delineating the molecular pathogenesis of HA MRSA Vs CA MRSA.

Preliminary assessment of bacterial diversity of Indonesian naturally-fermented milk products by pyrosequencing

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This work represents the first study of bacterial communities in the Indonesian naturally-fermented milk products, dadih and dangke. Dadih is manufactured using unpasteurized buffalo milk, after which it is fermented for three days. To produce dangke, heat-treated cow or buffalo milk is coagulated using papaya latex and then the curd is pressed (without maturation). Pyrosequencing of tagged amplicons of the V1-V3 regions of the 16S rDNA and Operational Taxonomic Unit-based (OTU-based) analysis were applied to obtain an overall approximation of the bacterial composition in dadih and dangke; and secondly, to elucidate possible differences between fermentation periods of dadih and dangke production [cow milk: A, and buffalo milk: B and C].

Pyrosequencing analysis revealed a high bacterial diversity in both products. Three phyla (bacteroidetes, firmicutes and proteobacteria) were identified in both products, and 18 genera from dadih and 19 genera from dangke were defined. In dadih, Lactococcus (22.9 – 60.7% of sequences) was the predominant genus, followed by Enterobacteriaceae and Acinetobacter. The abundance of Lactococcus throughout the fermentation stages increased remarkably, with a concomitant decrease in the number of sequences belonging to the Enterobacteriaceae family, while Acinetobacter abundance remained constant. Dadih prepared from cow’s milk revealed a diverse bacterial diversity (producer A). OTU analysis identified a set of taxa [Lactococcus, 20.5%; Lactobacillus, 18.5%; Streptococcus, 17.1%; Enterococcus, 15.3%; Leuconostoc, 9.7%; and unclassified genera belonging to the Enterobacteriaceae family, 6.9%] that would represent the core components of dangke sourced from cow’s milk. The Enterobacteriaceae family was the predominant taxa in dangke-producer B and C. Enterococcus (0.1% of sequences) was the only lactic acid bacteria detected in dangke-producer B; meanwhile Lactococcus (13.3% of sequences) and Enterococcus (0.5% of sequences) were identified in producer C. The role of lactic acid bacteria is important, not only in developing product quality, but also in improving product safety. Although taxa belonging to important milk-borne diseases were not found, a detailed understanding of the Enterobacteriaceae family associated with the fermentation process is required.