Programme and Abstract Booklet

6th Midlands Molecular Microbiology Meeting (M4) 2019

September 9/10th 2019

Venue:

West Atrium and Auditorium of the Jubilee Conference Centre

Triumph Road, Nottingham, NG7 2TU

University of Nottingham

https://www.nottingham.ac.uk/conference/fac-mhs/lifesciences/m4/index.aspx

#nottinghamM4

#ECMForum

#ECRchat

Programme at a Glance

Jubilee Conference Centre

University of Nottingham

Day	Time	Session
Sept 9th		*Polymicrobial interactions and microbial crowdsourcing*
	9.00 -10.30	Session 1
	10.30-11.15	Coffee with posters
	11.15-12.15	Session 2
	12.15-13.15	Lunch with posters
	13.15-15.15	Session 3
	15.15-16.00	Coffee with posters
	16.00-17.30	Session 4
	17.30-18.30	Early Career Researcher Led Panel Discussion 'Career Paths for microbiologists'
	19.00	Meal in Catering Atrium followed by Band in Newark Hall Bar
Sept 10th		*Signalling, virulence and antimicrobial resistance*
	9.00-10.30	Session 5
	10.30-11.15	Coffee with posters
	11.15-12.15	Session 6
	12.15-13.15	Lunch with posters
	13.15-15.15	Session 7
	15.15	Coffee with posters
	16.00-17.00	Session 8
	17.00	Open Lecture: Antimicrobial resistance
	18.00-19.00	Closing remarks and Wine Reception in West Atrium

Sessions 1-8 will be located in the Auditorium
Coffee, poster and wine reception will be located in the West Atrium

Contents:

Welcome and Background to M4 Conference

On behalf of the many people involved in the organization of this conference and the sponsors who have provided the financial support, you are welcomed to the 2019 Midlands Molecular Microbiology Meeting in Nottingham, affectionately known as the M4 conference.

The M4 meetings were started in 2014 to strengthen interactions and create opportunities for collaboration between Midland universities. The first M4 (Midlands Molecular Microbiology Meeting) was held at the University of Birmingham in September 2014, followed by the University of Nottingham (2015), University of Leicester (2016), University of Birmingham (2017) and last year at the University of Warwick (2018). In practice, there continues to be an audience drawn from further afield who attend the M4 meeting, attracted by the high quality science presented and convivial atmosphere.

This two day conference will provide talks and posters of applied, cutting-edge, interdisciplinary microbial research. This year's meeting will focus on cross-disciplinary talks covering applied and fundamental aspects of antimicrobial resistance and evolution, especially those within microbiomes. The sessions will cover microbial pathways which underpin cell envelope biogenesis, gene regulation, virulence in animal and plant hosts, plus biofilms. All of these processes offer novel antimicrobial targets, or can be exploited by industry.

The rationale of the meeting is to ensure that early career researchers (ECRs), at pre- and post doctoral levels, have the opportunity to promote their work to enable them to develop a network of researchers with related interests. Therefore, the majority of the talks have been selected from offered abstracts with the emphasis being placed upon the participation of ECRs. Poster sessions are a key feature of the meeting to ensure all attendees have the maximum opportunity to discuss their work with leading and new investigators in a socially conducive environment. To provide exposure to the poster presenters, each has been invited to deliver a 3 minute 'Flash' presentation to motivate the conference participants to engage broadly in research discussions. ECRs have also been invited to act as co-chairs, and have chosen the topic and constitution of a discussion panel that addresses an important concern, Career Options in Microbiology.

We are also delighted to welcome the winner of the Junior Awards in Microbiology series (University of Birmingham, www.jmtalks.com) to give a talk at this meeting.

We envision that this meeting will be important for networking with the aim to encourage increased collaboration, which is essential for high impact research. So, we encourage you all to ask questions in sessions and approach each other and discuss science.

To facilitate this, we have included a social evening on the first day with a meal followed by an informal evening entertained by a band that includes some of our scientific colleagues. We will also end the proceedings with an open wine reception in the elegant settings of the Jubilee Conference Centre.

If you have travelled from beyond the Midlands, we welcome you especially. We hope you will find new collaborators and strengthen existing interactions.

Finally, and very importantly, we thank all our sponsors for the support that they have provided to make this meeting possible. We hope the representatives that are in attendance will enjoy engagement with the community.

We hope you will be able to share excellent science, build networks and that the momentum of the M4 will continue for many years to come.

Full Programme

Day	Time	Session
Sept 9th		***Polymicrobial interactions and microbial crowdsourcing***
Session 1: CHAIR Paul Williams and ECR Co-chair Fiona Whelan		
Sponsored Speaker	9.00	**Invited speaker 1: James McInerney (University of Nottingham)** **Pangenomic models and co-occurrence**
	9.30	10 Flash posters (3 mins each) **2,4,6,8,10,12,14,16,18,20**
	10.00	Offered oral: Augustinas Silale (University of Oxford) **Characterisation of the bacterial DNA transporter involved in natural transformation**
	10.15	Offered oral: Jonathan Humphreys (University of Nottingham) **Clostridial strain degeneration: new approaches to an old problem**
	10.30	**Coffee with posters All even numbers**
Session 2: CHAIR Laura Hobley and ECR Co-chair Jack Leo		
	11.15	Offered oral: Robeena Farzand (University of Leicester**) ICEKp2: description of an integrative and conjugative element in *Klebsiella pneumoniae*, co-occurring and interacting with ICEKp1**
	11.30	Offered oral: Satpal Chodha (University of Sheffield) **Molecular patterning of *Vibrio cholerae* chromosome segregation proteins**
	11.45	10 Flash posters (3 mins each) **1,3,5,7,9,11,13,15,17,19**
	12.15	**Lunch with posters All odd numbers**
Session 3: CHAIR Jessica Blair and ECR Co-chair Shaun Robertson		
Sponsored Speaker	13.15	**Invited speaker 2: Lindsay Hall (Quadram Institute, Norwich UK)** **Early life microbiota-host interactions**
	13.45	10 Flash posters (3 mins each) **22,24,26,28,30,32,36,38,42**
	14.15	Offered oral: Lillie Purser (University of Leicester) **The role of air pollution and bacteria in COPD**
	14.30	Offered oral: Sonali Singh (University of Nottingham) ***Pseudomonas aeruginosa* Biofilm-associated Polysaccharide Psl Modulates Immunity through Engagement of C-type Lectin Receptors**
Sponsored Speaker	14.45	**Invited speaker 3: Simon Foster (University of Sheffield)** **Microbial Crowdsourcing: *Staphylococcus aureus* Infection Dynamics**
	15.15	**Coffee with posters All even numbers**

Session 4: CHAIR Paul Hoskisson and ECR Co-chair Jo Purves		
	16.00	Offered oral: Marwa Hassan (University of Warwick) **Predicting antibiotic persistence and associated virulence in *Pseudomonas aeruginosa* biofilms from an *ex-vivo* perspective**
	16.15	Offered oral: Ana da Silva (University of Nottingham) **Understanding Phenotypic Diversity of *P. aeruginosa* in Chronic Diabetic Ulcers**
	16.30	10 Flash posters (3 mins each) **21,23,25,27,29,31,33,35,37,39**
MICROBIOLOGY SOCIETY Sponsored Speaker	17.00	**Invited speaker 4: Lesley Hoyles (Nottingham Trent University)** **Microbiomes investigated by microbiomics, transcriptomics and metabolomics**
sfam society for applied microbiology Sponsored session	17.30	Early Career Researcher Led Panel Discussion '**Career Paths for microbiologists**' Invited Panellists: Lindsay Hall, Quadrum Institute (Academic) Ben Swift, PDP Biotech (Biotech) Jo Slater-Jeffries, National Biofilm Innovation Centre (Knowledge transfer) Rachael Pearson, University of Nottingham (Public engagement) Chairs: Sonali Singh and Colman O'Cathail
azotic TECHNOLOGIES Sponsored session	**18.30**	**Close and Meal in Catering atrium followed by Band in Newark Hall Bar**
Sept 10th		***Signalling, virulence and antimicrobial resistance***
Session 5: CHAIR Luisa Martinez-Pomares and ECR Co-chair Manuel Romero		
MICROBIOLOGY SOCIETY Sponsored Speaker	**9.00**	**Invited speaker 5: Petra Dersch (University of Münster, Germany)** **RNA regulators in gastrointestinal bacteria**
	9.30	20 Flash posters (3 mins each**)** **41,43,45,49,51,53,55,57,59,61,63,65,67,69,71,73,75,77,79,81**
	10.30	**Coffee with posters All odd numbers**
Session 6: CHAIR Marco Oggioni and ECR Co-chair Michelle Buckner		
	11.15	17 Flash posters (3 mins each) **44,46,48,50,52,54,56,58,60,62,64,66,70,74,76,78,82**
	12.15	**Lunch with posters All even numbers**
Session 7: CHAIR Tim Knowles and ECR Co-chair Ling Chin Hwang		

National Biofilms Innovation Centre Sponsored International Frontier Lecture	**13.15**	**Invited speaker 6: Rikke Meyer (Aarhus University, iNANO, Denmark)** **Fundamental Mechanisms of bacterial adhesion and biofilm formation**
	13.45	Offered oral: Louise Corscadden (University of Leicester) **Air pollution differentially affects the *Staphylococcus aureus* oxidative stress response**
	14.00	Offered oral: Josh McQuail (Imperial College, London) **An unusual feature in the subcellular landscape of long-term nitrogen starved *Escherichia coli***
	14.15	Offered oral: Rebeca Bailo (University of Birmingham) **Path-seq identifies an essential mycolate remodeling program for mycobacterial host adaptation**
	14.30	Offered oral: Robert Cogger-Ward (University of Nottingham) **A conserved protein, BcmA, mediates motility, biofilm formation, and host colonisation in Adherent Invasive *Escherichia coli***
INTERFUTURE Sponsored Speaker	**14.45**	**Invited speaker 7: Jake Malone (John Innes Centre, Norwich)** **Bacterial signalling during plant-microbe interactions**
	15.15	**Coffee with posters All odd numbers**
Session 8: CHAIR Sam McLean and Co-chair Stephan Heeb		
	16.00	Offered oral: Michelle Buckner (University of Birmingham) **Discovery of compounds which reduce transmission of antimicrobial resistance plasmids**
	16.15	Offered oral: Joseph Wanford (University of Leicester) **Hypervirulent, and MDR lineages of *Klebsiella* replicate in macrophages *in vitro*, in murine organs, and in an *ex vivo* model of porcine liver and spleen co-perfusion**
	16.30	Offered oral: Rebekah Jones (University of Warwick) **An intriguing link between membrane lipid remodelling and antimicrobial resistance in pathogen *Pseudomonas aeruginosa***
	16.45	Offered oral: Junior Awards in Microbiology (JAM) finalist Liam Rooney, (University of Strathclyde) https://thejamtalks.com/ **Mesoscopic microbiology exploring the inner workings of *E. coli* biofilms using the Mesolens**
sfam society for applied microbiology Sponsored Speaker	**17.00**	**Open Lecture Invited speaker 8: Fernando Baquero (Ramón y Cajal University Hospital, Madrid)** **Individual-Health, One-Health and Global-Health in Antibiotic Resistance**
University of Nottingham UK | CHINA | MALAYSIA Sponsored open reception	**18.00-19.00**	**Closing remarks and Wine Reception** **Registration through Eventbrite is free, but not included in the M4 meeting registration. Please use the link below to register.** https://www.eventbrite.co.uk/e/open-lecture-by-professor-fernando-baquero-tickets-68592075745

Organizing Committee

- **Steve Atkinson,** University of Nottingham
- **Colman O'Cathail,** University of Nottingham (ECR)
- **Miguel Camara,** University of Nottingham
- **Sarah English,** University of Nottingham (Logistics)
- **Kim Hardie,** University of Nottingham (CONFERENCE CHAIR)
- **Stephan Heeb,** University of Nottingham
- **Phil Hill,** University of Nottingham
- **Laura Hobley,** University of Nottingham
- **Joe Ingram,** University of Nottingham (ECR)
- **Michael Loughlin,** Nottingham Trent University
- **Luisa Martinez-Pomares,** University of Nottingham
- **Samantha Mclean,** Nottingham Trent University
- **David Negus,** Nottingham Trent University
- **Shaun Robertson,** University of Nottingham (ECR)
- **Manuel Romero,** University of Nottingham (ECR)
- **Sonali Singh,** University of Nottingham (ECR)
- **Paul Williams,** University of Nottingham

Supporting Volunteers

- Charlotte May
- Natalia Romo Catalan
- James English
- Jill Reid
- Birte Blunk
- Mark Perkins
- Carol Paiva
- Ewan Murray

Awards

Microbiology Society Journal of Medical Microbiology Poster Prize:

There will be two poster prizes for the top poster presentations

Each winner will receive a cash prize of £50 and a certificate.

Microbiology Society Journal of Medical Microbiology Oral Presentation Presentation Prize:

There will be two poster prizes for the top 15 minute oral presentation

Each winner will receive a cash prize of £100, a one-year membership of the Microbiology Society, and a certificate.

Practical Information

For Oral Presenters:

All speakers (including the discussion panel members) are requested to provide their slides either before the first talk of the morning or during lunch on the day that they are due to present. There will be someone at the lectern in the auditorium to help with this.

Slides should be formatted for widescreen: 16:9

Invited speakers are allocated 30 minutes for their talks (25 min + 5 Min Q&A).

Offered talks are allocated 15 minutes for their talks (12 min + 3 Min Q&A). There is no poster board associated with offered talks.

In the event that you would like to use your own computer for your talk, please bring along the cables that are required to connect your PC or Mac in case the audio-visual team do not have the appropriate connections.

For Poster Flash Poster Presentations

Flash Poster presenters are allocated a strict 3 min for their talk without any Q&A. It is important that slides are provided a minimum of three working days before the conference ie. **by 3rd September** since they will be combined into a single powerpoint file to ensure swift change overs. No more than 2 slides will be allowed per presenter. The chairs will enforce the timing of this session precisely, and to enable seamless presenter exchanges, we request that presenters form a line in order of delivery by standing along the side of the auditorium.

For Poster Board Presentations

All poster boards will have a number that corresponds with your abstract ID. Please locate this number on the boards and hang your poster at the beginning of the first day during registration. Please attend your poster

during the timeslots assigned in the programme. Posters should be removed from the boards by 18.00 on September 10th, 2019. Posters on boards after this time will not be returned to presenters. The organisers are not responsible for posters left on the boards or poster containers left unattended.

Poster dimensions:

Please prepare your poster according to the following guidelines:

Portrait style, **A0** size maximum. Measurements should not exceed a height of 1189mm (46.8 inches) and a width of 841mm (33.1 inches). (Poster boards are 2.1m high x 0.92m wide; 7ft high x 3ft wide).

Badges

Badges must be worn at all times for security purposes

Contact Information:

The University of Nottingham
University Park
Nottingham
NG7 2RD

Email: LS-M4Nottingham@exmail.nottingham.ac.uk

Dietary requirements

Every effort will be made to meet all dietary requirements. The information has been passed to the catering team in advance.

Drinks reception on day 2

Drinks will be served after the closing remarks in the West Atrium of the Jubilee Conference Centre until 19.00. For catering purposes, you are requested to indicate that you will be attending via the Eventbrite link below. There will be no charge, courtesy of financial support from the University of Nottingham.

https://www.eventbrite.co.uk/e/open-lecture-by-professor-fernando-baquero-tickets-68592075745

Evening meal on 9th September

The evening meal will be served at 19.00 on September 9th 2019 in the Catering Atrium on the University of Nottingham Jubilee Campus. Sponsorship from Azotic has enabled us to provide some wine to accompany your meal and you will be able to purchase additional drinks if required from the foyer bar in the Jubilee Conference Hotel or Newark Hall bar.

The band 'The FLOW' comprised of Phil Hill, Cameron Alexander, Robin Hall and Gayle Straw will play two sets in the bar of Newark Hall on Jubilee Campus between 20.30 and 22.30 on September 9th 2019. There will be a cash bar for refreshments (8 p.m. to 11 p.m.).

http://theflow.rocks/

Emergency contact information

Sarah English. LS-M4Nottingham@exmail.nottingham.ac.uk

Kim Hardie. Kim.Hardie@nottingham.ac.uk

Getting to the Conference Venue

Maps to the conference venue and other access information can be found from the links below.

https://www.nottingham.ac.uk/about/visitorinformation/mapsanddirections/jubileecampus.aspx

https://www.nottingham.ac.uk/sharedresources/documents/mapjubileecampus.pdf

Parking is by **Printed Event Voucher only**, if you still require a permit please contact LS-M4Nottingham@exmail.nottingham.ac.uk with your registration details.

The University of Nottingham operates a car parking policy which requires all guest cars to display a parking voucher between 09:15-16:00 on weekdays and for cars to be parked in Orange Parking Zones only.

Display your parking voucher face-up in a prominent position on the inside of the windscreen where all details can be clearly read from the outside of the vehicle. Please note, failure to display a voucher as detailed will result in a civil parking notice being issued.

The parking voucher is valid only for the event on the date(s) shown and for the Vehicle Registration, the Event Title and Parking Code detailed on the face. Alternatively, you can purchase a ticket and park in the designated 'Pay and Display' zones.

The issue of a parking voucher does not guarantee the availability of a parking space and must be used in accordance with any guidance issued by the Security office.

Registration

Upon arrival, please register at the Welcome desk and receive a copy of the full programme and your badge. To protect the environment, we will not be printing this Conference Abstract Book which will be available online on the website for you to download in advance and print out at your convenience. Registration is open from **08.15** on 9th.

https://www.nottingham.ac.uk/conference/fac-mhs/lifesciences/m4/index.aspx

Session Etiquette

Whilst you are in a session, please mute all mobile phones and other electronic devices.

Social Media

We welcome live tweeting and sharing by other social media as a means to disseminate information to the broader scientific community who are not in attendance, and to increase the visibility of the science communicated by our presenters.

Please use hashtag **#nottinghamM4** and include **@MicrobioSoc**, **@UniofNottingham, @ukbiofilms, @SfAMtweets, #JMedMicro**

We recognise that some presenters might consider their data to be sensitive, and if they do not wish details from their presentations to be shared we ask presenters to make an announcement to this effect at the beginning of their presentation or put a notification on their poster.

WIFI

Free wifi obtainable by logging in to the Jubilee Conference Centre network. No password required.

Accommodation at Newark Hall

9th September: Rooms available for check-in from **3 p.m.**

10th September: Full English or Continental Breakfast available in the Catering Atrium from **7 a.m. to 8.30 a.m.**

Check-out of rooms by **10 a.m**. on 10th September.

Accommodation at Jubilee Conference Centre

Rooms will be available from **2 p.m.** on the first day of your reservation.

Breakfast will be served in the Spokes Bar, Jubilee Conference Centre from **7 a.m. to 8.30 a.m.**

Check-out: By **11 a.m.** on the day of departure

Abstracts and Biographies:
Invited Speakers

Professor James McInerney

University of Nottingham, UK

http://mcinerneylab.com/james-mcinerney/

Biography: James McInerney is Head of the School of Life Sciences at The University of Nottingham. He is an elected Fellow of the American Academy of Microbiology. James' BSc and PhD were awarded by University College Galway and subsequently he did a post-doc at The Natural History Museum. In 1999 he set up the bioinformatics research group at NUI Maynooth. For the academic year 2012-2013, he took a sabbatical at the Center for Communicable Disease Dynamics at Harvard University. In 2013 he was awarded a DSc by the National University of Ireland. James was Chair in Evolutionary Biology at The University of Manchester and the Director for the research domain of "Evolution, Systems and Genomics" at the University of Manchester from 2015-2018. He was elected Fellow of the American Academy of Microbiology in 2015 and Fellow of the Linnean Society in 2016. He has served as an Associate Editor of Molecular Biology and Evolution, and the Journal of Experimental Zoology. He is a scientific associate of The Natural History Museum, London.

Abstract: The genomes of different individuals of the same prokaryote species can vary widely in gene content, displaying varying proportions of core genes, which are present in all genomes, and accessory genes, whose presence varies between genomes. Together these core and accessory genes make up a species' pangenome. It is likely that this extensive diversity in gene content is due to natural selection, combined with variation in ecological features. However, there is an ongoing debate about the contribution of accessory genes to fitness. We developed a mathematical model to simulate the gene content of prokaryote genomes and pangenomes. Our model focuses on testing how the fitness effects of genes and their rates of gene gain and loss would affect pangenome properties. I will then outline our approach to identifying interactions between accessory genes in pangenomes and how we find significant co-occurrence of some genes with one another and also significant avoidance of genes with one another.

Dr Lindsay Hall

Quadram Institute, UK

https://quadram.ac.uk/lindsay-hall/

Biography: Dr Lindsay Hall is the Microbiome Group Leader at the Quadram Institute, Norwich. She has a BSc (Hons) in Microbiology from the University of Glasgow, a PhD in Microbiology and Immunology from the University of Cambridge (Sanger Institute), was a postdoctoral fellow at the APC Microbiome Institute (University College Cork, Ireland), and a Senior Lecturer at the University of East Anglia. Lindsay is also a Wellcome Trust Investigator and her research team studies early life gut microbiota-host interactions with a particular focus on the beneficial genus *Bifidobacterium*

Abstract: The gut-associated microbiota is essential for multiple physiological processes, including immune system development. Acquisition of our initial pioneer microbial communities, including the dominant early life genus *Bifidobacterium*, occurs at a critical period of immune maturation and programming. Bifidobacteria are resident microbiota members throughout our lifetime and have been shown to modulate specific immune cells and pathways. Notably, reductions in this genus have been associated with several diseases, including inflammatory bowel disease. Here I will discuss our research which explores the role of the early life microbiota and *Bifidobacterium* in driving 'normal' immune response development via specific cross-talk mechanisms, and how *Bifidobacterium* can also used to beneficially modulate inflammatory and pathological epithelium responses during disease via specific bifidobacterial factors and engagement with host cell receptors, thus modulating transcriptional cascades and regulatory networks. These data highlight the importance of maintaining healthy homeostatic cross-talk with specific microbiota members and opens up mechanistic pathways that could be targeted for future therapies.

Professor Simon J Foster

University of Sheffield, UK

https://www.sheffield.ac.uk/mbb/staff/simonfoster/simonfoster

Biography: Simon Foster is the Director of the Florey Institute, with 2 main areas of microbiological research that he has been pursuing for more than 20 years:

- **Bacterial cell architecture and dynamics:** The shape and integrity of bacteria are determined by the cell wall. The synthesis of the cell wall is also the site of action of important antibiotics such as penicillin. His laboratory takes an interdisciplinary approach to investigate the architecture of the cell wall and its dynamics during growth and division. This encompasses the use and development of novel high-resolution microscopy techniques.

- ***Staphylococcus aureus* stress resistance , pathogenicity and the identification of new vaccine targets:** *S. aureus* is a major human pathogen of increasing importance due to the spread of antibiotic resistance. His research spans from the fundamental through to translational approaches to develop new control regimes. His group employs the use of several models to understand the basis of host:pathogen interaction and the dynamics of infection.

Abstract: *S. aureus* is an important human pathogen of increased significance due to the spread of antibiotic resistance. If we are to combat the scourge of antimicrobial resistance, we must understand how *S. aureus* causes disease and how we can use this information to better develop new control regimes. We have been examining the dynamics of *S. aureus* disease from the point of infection through to outcome. This has revealed a complex interplay between the pathogen and the host innate immune system, with immune bottlenecks, pathogen population containment and expansion. We have also found the native human microflora to play an important role in *S. aureus* infection creating a *ménage a trois*, the outcome of which is dependent on multiple factors. Unravelling this process provides new avenues for the design of approaches to prevent and treat infection.

Dr Lesley Hoyles

Nottingham Trent University, UK

https://www.ntu.ac.uk/staff-profiles/science-technology/lesley-hoyles

Biography: Dr Lesley Hoyles is an Associate Professor in Microbiology in the Department of Biosciences at Nottingham Trent University. She undertook a BSc in Microbiology (1997) and PhD (2009) in Gut Microbiology at the University of Reading. She also completed an MSc in Bioinformatics and Theoretical Systems Biology (2012) at Imperial College London. From 2009 to 2011 she was a Government of Ireland Postdoctoral Fellow in Science, Engineering and Technology at University College Cork; from 2014 to 2016 she was an MRC Intermediate Research Fellow in Data Science at Imperial College London. She is a member of the ESCMID Study Group for Host and Microbiota Interaction (ESGHAMI), and serves on the editorial boards of *PeerJ* and *FEMS Microbiology Letters*. Her research interests encompass bacterial taxonomy, anaerobic bacteria, microbial metabolites and microbial ecology. Since training as a bioinformatician, Dr Hoyles has worked extensively in the field of systems biology, working on numerous animal and human studies integrating microbiomic, metabolomic and transcriptomic data to better understand how the microbiome and its metabolites influence intestinal and systemic health. Using integrated systems-level approaches, Dr Hoyles has defined the contribution of the microbiome and its metabolites to non-alcoholic fatty liver disease, metabolic retroconversion and integrity of the blood–brain barrier.

Abstract: Alongside our improved understanding of the composition and function of the human gut microbiome has come the realization that the microbes harboured in our intestinal tract contribute to host metabolism – the so-called microbe–mammalian co-metabolic axis – and influence immune function, metabolic homeostasis and health. *In vivo* studies involving groups of non-alcoholic fatty liver disease and type 2 diabetic patients have used integrated omics approaches to demonstrate the phenotypic effects of microbiome-associated metabolites in the context of human metabolic diseases, and have produced mechanistic data to support findings. Integrative analyses of microbiomic, metabolomic and immunological data have identified microbial, biochemical and host factors central to dysregulation in inflammatory bowel disease. Several rodent studies have shown how microbiome-associated metabolites influence the gut–brain axis and the gut–liver axis. In addition, *in vitro* and *in vivo* studies have demonstrated direct interaction of these metabolites with genes encoded by the liver and brain. This talk will highlight how the aforementioned studies have used a variety of approaches (i.e. combining one or more of microbiomics, metabolomics, transcriptomics, proteomics) to move microbiome research beyond simple correlation-based analyses to complex systems-level studies to better understand host–microbiome interactions in relation to health and disease.

Prof Dr Petra Dersch

Institute for Infectiology, University of Münster, Germany

https://www.medizin.uni-muenster.de/en/faculty-of-medicine/about-us/people-awards/details/?uid=210&L=1

Biography: Petra Dersch graduated in Biology at the University of Konstanz. She continued her studies of Microbiology at the University of Konstanz and the Max-Planck-Institute for Terrestrial Microbiology Marburg and finished her PhD in 1995. After that, she worked as a postdoc in the laboratory of Dr. Ralph Isberg at the Tufts Medical School/Howard Hughes Medical Institute in Boston/USA. In 1998, back in Germany, she started as a Research Assistant with her own group at the Freie Universität Berlin. From 2003 - 2005, Petra Dersch was Junior Research Group Leader at the Robert Koch Institute Berlin. In 2005, she was appointed at the Technische Universität Braunschweig as Associate Professor in microbiology. From 2008 to 2019, Petra Dersch was Head of the Department of Molecular Infection Biology at the Helmholtz Centre for Infection Research in Braunschweig and holded a co-appointment as full professor in microbiology and infection biology at the Technische Universität Braunschweig. Since February 2019, she is the head of the Institute of Infectiology in the Center for Molecular Biology of Inflammation (ZMBE) of the University Münster. Her main research field is molecular pathogenesis of enteric bacterial pathogens.

Abstract: RNA regulators in gastrointestinal bacteria
Gastrointestinal pathogens such as *Yersinia pseudotuberculosis* evolved numerous strategies to survive in environmental reservoirs and mammalian hosts. A hallmark is the ability to rapidly adjust the lifestyle upon host entry to prevent attacks by the host immune systems. The pathogen employs a plethora of control elements to fine-tune regulatory networks. To capture the range, magnitude and complexity of the underlying control mechanisms, we used comparative RNA-seq-based transcriptomic profiling under infection-relevant conditions *in vitro* and during the infection process in mice. We identified riboswitch-like RNA elements, a set of antisense RNAs, and previously unrecognized *trans*-acting RNAs, which are differentially regulated under infection conditions. We revealed a temperature- and host-induced reprogramming of important metabolic pathways, virulence traits, and discovered CRP as master regulator of non-coding RNAs. Individual regulatory RNAs, which are differentially regulated during infection were characterized and their role in infection was elucidated using mouse infection models. Among the regulatory RNAs, which are most important for *Yersinia* virulence, are the Crp-dependent Csr-type regulatory RNAs found to control multiple virulence-relevant metabolic processes. Our finding highlights a novel level of complexity in which the concerted action of transcriptional regulators and non-coding RNAs adjusts the control of *Yersinia* fitness and virulence to the requirements of their virulent life-style.

Dr Rikke L Meyer

Aarhus University, Denmark

https://inano.au.dk/about/research-groups/biofilm-group-assoc-prof-rikke-meyer/

Biography: Rikke Meyer is Associate Professor at Aarhus University, Denmark, where she is heading an interdisciplinary research group at the Nanoscience Center, iNANO. Her research over the past 15 years has focused on understanding how bacteria form antibiotic-tolerant biofilms, and she has worked with chemists and material scientists to transfer that knowledge into development of materials, coatings, or new treatment methods for combatting biofilms in both industrial and clinical settings.

Abstract: Bacteria deploy a large array of specific- and non-specific interactions to attach to abiotic materials and initiate biofilm formation. These mechanisms vary greatly between species, and each species typically possess several different mechanisms that are used in different circumstances. Only one adhesin appears to be ubiquitous in the microbial world: DNA. In this talk, I will discuss of some of the basic mechanisms in bacterial attachment to abiotic surfaces, using DNA as an example of a non-specific adhesin, and surface-bound proteins as examples of highly specific adhesins. The diversity in attachment mechanisms makes it challenging to develop a one-fits-all solution to prevent biofilms. I will discuss how fundamental knowledge about bacterial attachment can be used in the development of materials that are less susceptible to biofilm formation.

Dr Jake Malone

Senior Lecturer, UEA/John Innes Centre, UK

https://www.jic.ac.uk/people/jacob-malone/

Biography: From the start of my career, I have been fascinated by how bacteria sense and understand changes in their environment, and respond by altering their behavior. My research career; from my PhD in the Plant Sciences Dept. at Oxford University (UK), through my post-doctoral work in the Biozentrum in Basel, Switzerland, to my current position as a Project Leader at the John Innes Centre (jointly with the University of East Anglia, UK, since 2011), has focused on understanding how different *Pseudomonas* species sense their environments and interact with plants and humans. My lab uses a combination of molecular microbiology, biochemistry and population genetics/environmental microbiology to investigate how *Pseudomonas* colonise and infect their hosts, and the implications of this for plant health and disease.

Abstract: *Pseudomonas* species employ complex, dynamic signalling networks to fine-tune responses to changing environments, with regulation taking place at the transcriptional, post-transcriptional and post-translational levels. Control of mRNA translation and hence protein abundance is a crucial element of this regulatory network. As part of our ongoing research into the signalling pathways that control plant colonisation by *Pseudomonas* spp., we identified the ribosomal modification protein RimK, which influences the transition between active and sessile bacterial lifestyles.

RimK is an ATP-dependent glutamyl ligase that adds glutamate residues to the C-terminus of ribosomal protein RpsF. This in-turn induces specific changes in ribosome function and translational output. RimK activity is itself under complex, multifactorial control; by the bacterial second messenger cyclic-di-GMP, a phosphodiesterase trigger enzyme (RimA), and a polyglutamate-specific protease (RimB). Deletion of the *rim* operon affects phenotypes including attachment, motility and cytotoxicity, severely compromising rhizosphere colonisation by the soil bacterium *Pseudomonas fluorescens.*

Using a combination of protein biochemistry, quantitative proteomics and ribosomal profiling experiments, we examined the relationship between ribosomal modification and downstream changes in the *P. fluorescens* proteome. RimK activity leads to active proteome remodelling by two main routes; indirectly, through changes in the abundance of the global translational regulator Hfq and directly, with translation of surface attachment factors, amino acid transporters and key secreted molecules linked specifically to RpsF modification. Our findings support the conclusion that post-translational ribosomal modification represents a novel signalling mechanism that rapidly tunes global gene translation and protein abundance in response to environmental signals.

Professor Fernando Baquero

Ramón y Cajal University Hospital, and Ramón y Cajal Institute for Health Research, Madrid, Spain.

Biography: Fernando Baquero, MD, Ph.D., directed the Department of Microbiology at the Ramón y Cajal University Hospital (1977-2008); from 2008 to date, he has been Scientific Director of the Ramón y Cajal Health Research Institute (IRYCIS), and currently Research Professor in Microbial Evolution in the IRYCIS. He was Senior Scientist in Evolutionary Biology at the Center for Astrobiology (CAB, INTA-NASA). He received the Excellence Awards of the American ASM-ICAAC, the European and Spanish Societies for Clinical Microbiology and Infectious Diseases; the Descartes Award for International Cooperative Research of the EU Commission, the Garrod Award and Medal of the British Society for Antimicrobial Chemotherapy, the André Lwoff Award of the European Federation of Microbiological Societies (2015), and the Arima Award for Applied Microbiology of the International Union of Microbiological Societies (2017). He is member of the American Academy of Microbiology, the European Academy for Microbiology, the European Academy for Clinical Microbiology and Infectious Diseases, and the Ibero-American Academy for Evolutionary Biology. He has a Google Scholar H-index of 100 (38,000 citations) and 497 publications referred in PubMed database.

Abstract: Studies on antimicrobial resistance (AMR) have a huge heuristic value for illustrating principles and generating hypotheses that guide human activity. The global spread of resistant organisms caused by our actions (for example, the use of antibiotics and general pollution) and inactions (for example, lack of proper sanitation) shows how a defined and measurable biological risk, influencing both our health and the health of the planet. Along little more than the last decade, the focus on antibiotic resistance has shifted from individual health (risks for the infected individual, first, in hospitals, then, in the community), to One-Health and Global-Health. One Health focuses on the role of interconnected (and hence geographically close) ecosystems in the emergence and dissemination of AMR, typically among humans and animals at the local level, and foster the implementation of integrated interventions for fighting AMR at, say, the city or regional level. Global health, in contrast, addresses the global conditions that facilitate the worldwide spread of AMR, and emphasizes that AMR is not only a problem for patients, but for the health of global human and animal communities, and probably for the health of the planet. Antibiotics are in the environment. In combination with many other environmental challenges, the entire microbiosphere of the planet is at risk. The Global Health approach emphasizes that the control of AMR requires integrated political and socioeconomic actions to be taken by countries, international organizations and other actors on the global stage.

Early Career Researcher Led Panel Discussion

"Career paths for microbiologists"

Career options are an important consideration for those in the early stages of their career, whether that be studying for a PhD, or working as a postdoc. This session will include a panel of 4 members, all of whom have a PhD and you have each followed very different career trajectories since their PhD. Each panellist will present a brief overview of their career to date, before the session is opened to questions from the audience, which can be anything related to career development.

ECR co-chairs of the session: **Colman O'Cathail and Sonali Singh**

Panel biographies

Dr Lindsay Hall

Please refer to Speaker details above.

Dr Rachel Pearson

Rachael began her career as a trainee Biomedical Scientist for the Health Protection Agency, and following a string of 'superbug' outbreaks, developed a strong interest in the portrayal of science in the media. She went on to complete a PhD looking at the role of quorum sensing in *Pseudomonas aeruginosa* biofilms at the University of Nottingham, under the supervision of Professors Paul Williams and Miguel Camara. During this time, she became increasingly aware of the importance of effective engagement with research. Following her PhD, Rachael's first job was in the communications team at JISC, where she wrote and promoted a guide to online resources for health and life sciences. Rachael returned to the University of Nottingham in 2008 as a Researcher Training & Development Manager in the Graduate School coordinating and delivering public engagement training and opportunities for researchers. In March 2019, she was appointed as Head of Public Engagement at the University of Nottingham

Dr Ben Swift

Ben is currently Director of Research and Development for a University of Nottingham spin-out company called PBD Biotech as well as a Research Fellow of AMR at the Royal Veterinary College. He carried out his undergrad and PhD at the University of Nottingham in microbiology where he had a strong interest in the development of novel diagnostic tools for mycobacterial infections. This led to the development of novel technology and eventually a patent, which is being exploited commercially. Currently Ben is working on diagnostics for Tuberculosis in both animals and humans and has ongoing trials, collaborating with stakeholders in the UK and worldwide.

Dr Jo Slater-Jefferies

Jo joined NBIC as Operations Director in April 2018. Between 2011 and 2018 she was the General Manager for EpiGen, a global research consortium. Jo started her scientific career at Sciona Ltd, a spin-out company combining lifestyle data and genetic analysis, as a Senior Scientist and Quality Manager. Jo is a Director and Trustee of the Asthma, Allergy and Inflammation Research (AAIR) charity. Jo holds a BSc (Honours) in Molecular Biology and an industrial sponsored PhD from the University of Portsmouth, UK. In 2014, Jo received a MBA and in 2017 became a Chartered Manager at the Chartered Management Institute.

Abstracts: Offered Orals

9th September

OO1

Characterisation of the bacterial DNA transporter involved in natural transformation

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Natural transformation is a mode of horizontal gene transfer in bacteria, including many pathogenic species. It involves uptake of naked DNA from the environment. The mechanistic details of how specialised transport proteins allow passage of DNA trough the cell envelopes remain elusive. I am studying an essential component of the transformation machinery, ComEC, which is thought to form a pore for DNA transport through the cell membrane but is poorly characterised.

I performed an expression screen of ComEC homologues in *E. coli* in order to obtain recombinant protein for structural and biochemical analysis. Negative stain electron microscopy was used for obtaining structural data. Individual domains of the protein were also expressed, and one domain was characterised using biophysical and biochemical assays.

Low resolution structural data suggest that the two extramembranous domains of ComEC are located on the same side of the membrane, contrary to some previous biochemical data. This implies that they work together during transport. Thermofluor assays with the C-terminal domain of ComEC showed that it has a selective metal binding site. The domain also showed metal-dependent activity in nuclease and phosphoesterase assays. I am currently trying to verify these results *in vivo* in *Bacillus subtilis*.

The data allow proposition of a model for how ComEC transports DNA, which is consistent with previous reports that only one strand of DNA reaches the cytoplasm and also provides a plausible source of energy for transport.

OO2

Clostridial strain degeneration: new approaches to an old problem

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Industrial use of solventogenic *Clostridia* has focused on strain improvements through metabolic engineering but there has been little investigation into population dynamics. Here we examine social interactions of the solvent producing species *Clostridium beijerinckii* to investigate the evolutionary driving forces behind the phenomenon of strain degeneration. Degeneration is characterised by a reduction or complete loss in solvent and endospore formation occurring in both batch and continuous fermentations making it a serious problem for commercial applications.

To gain insights into the degeneration process we repeatedly sub-cultured *C. beijerinckii* NCIMB 8052 to promote the emergence of degenerates. Four colony morphologies were identified, characterised, with the most severe morphotype unable to form solvents and spores. We sequenced the genomes of 71 independent isolates to identify any genetic causes underlying degeneration. Four hotspots emerged which contained considerably more mutations than the rest of the genome. Targeted inactivation was employed to confirm the importance of these hotspots.

Social interactions were studied using wild type and a naturally evolved degenerate grown in various starting ratios. At low initial frequencies, it displayed a higher fitness than the wild type, but its fitness rapidly decreased at higher starting frequencies. This frequency-dependent fitness is suggestive of social cheating. The mutant was however unable to sustain itself in both monoculture and mixed culture during stationary phase, suggesting defects that could not be rescued by the wild type.

A combination of genetic, ecological and evolutionary approaches may be key to understanding and controlling the process of strain degeneration in industrial applications.

OO3

ICEKp2: description of an integrative and conjugative element in *Klebsiella pneumoniae*, co-occurring and interacting with ICEKp1

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Klebsiella pneumoniae is a human pathogen, prominent in antimicrobial-resistant and nosocomial infection. The integrative and conjugative element ICEKp1 is present in a third of clinical isolates and more prevalent in invasive disease; it provides genetic diversity and enables the spread of virulence-associated genes. We report a second integrative conjugative element that can co-occur with ICEKp1 in *K. pneumoniae*. This element, ICEKp2, is similar to the *Pseudomonas aeruginosa* pathogenicity island PAPI. We identified ICEKp2 in *K. pneumoniae* sequence types ST11, ST258 and ST512, which are associated with carbapenem-resistant outbreaks in China and the US, including isolates with and without ICEKp1. ICEKp2 was competent for excision, but self-mobilisation to recipient *Escherichia coli* was not detected. In an isolate with both elements, ICEKp2 positively influenced the efficiency of plasmid mobilisation driven by ICEKp1. We propose a putative mechanism, in which a Mob2 ATPase of ICEKp2 may contribute to the ICEKp1 conjugation machinery. Supporting this mechanism, *mob2*, but not a variant with mutations in the ATPase motif, restored transfer efficiency to an ICEKp2 knockout. This is the first demonstration of the interaction between conjugative genetic elements in a single Gram-negative bacterium with implications for understanding evolution by horizontal gene transfer.

OO4

Molecular patterning of *Vibrio cholerae* chromosome segregation proteins

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Chromosome segregation is an essential and dynamic process that has to be coordinated in space and time with cell division. In bacteria, this is orchestrated by the Par (Partition) system that localizes and moves the replicated chromosomes apart. Despite recent progress in our understanding of the segregation of smaller genomes such as plasmids, the molecular mechanisms underpinning chromosome segregation remain elusive. The Par system consists of *parS* centromere-like sequences and two proteins, ParA and ParB. In *Vibrio cholerae*, each of its two chromosomes has a distinct Par system to independently control its segregation. The Par system of chromosome 2 is essential for *V. cholerae* virulence and viability, making it a prime target for new antibiotics. We found that ParA2 exhibits an asymmetric concentration gradient in the cell that shows remarkable pole-to-pole oscillations. However, the molecular basis of these protein oscillations in positioning and segregation of chromosomes remain unclear. We show that ParA2 binds DNA cooperatively in the presence of ATP. Using cryo-EM, we determined the structure of ParA2-DNA filaments. Our map showed new interactions at the dimer-dimer interface spanning the entire length of the ParA2 dimer, providing structural basis of higher-order oligomeric assembly and DNA-binding cooperativity. We found that ParA2 undergoes a slow conformational transition between non-binding to DNA-binding states, regulated by its cognate partner, ParB2. This time delay allows ParA2 to migrate and rebind the chromosome at the poles, leading to the continuous pole-to-pole cycling that incrementally translocates the chromosomal loci toward opposite cell halves for segregation.

OO5

The role of air pollution and bacteria in COPD

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Air pollution is the single largest environmental health risk worldwide. Particulate matter (PM) air pollution is released as a result of fossil fuel combustion and vehicle motion, breaking and tyre wear. It has been shown that exposure to air pollution, specifically PM, can cause increased levels of respiratory disease, including the exacerbation of COPD, which is most frequently associated with bacterial infection. Despite this, the effects of air pollution exposure on COPD associated respiratory bacteria, such as *Haemophilus influenzae, Moraxella catarrhalis* and *Streptococcus pneumoniae* are largely unknown. Our recent publication was the first to document that as well as damaging the host, PM has a direct impact on bacteria that can cause respiratory infections. We showed that exposure to black carbon (BC), an important component of particulate PM air pollution, results in alterations in biofilm structure in both *Streptococcus pneumoniae* and *Staphylococcus aureus*, and increases dissemination of colonising *S. pneumoniae* in *in vivo* models.

Following on from this work, we aim to determine how BC impacts the growth, behaviour and virulence of key bacteria associated with the exacerbation of COPD, including non-typeable *Haemophilus influenzae*. Our current data show that BC exposure is decreasing the biofilm forming ability of NT*Hi* strains 162 and 375. In addition to this, pre-exposing NT*Hi*375 cells to BC, prior to infection of A549 cells, increases their ability to adhere to human epithelial cells. This suggests that the frequency of bacterial infection induced COPD exacerbation may be altered in patients from highly polluted areas.

OO6

Pseudomonas aeruginosa Biofilm-associated Polysaccharide Psl Modulates Immunity through Engagement of C-type Lectin Receptors

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Background: Chronic *Pseudomonas aeruginosa* (PA) infections cause morbidity and mortality in individuals with underlying conditions, and are characterised by drug and host-tolerant biofilm formation. The exopolysaccharides Psl and/or Pel are essential for PA biofilm formation, and can modify host immunity. However, the host receptors involved in PA exopolysaccharide recognition are unknown.

We hypothesised that Psl recognition by C-type lectin receptors (CLRs) Mannose Receptor (MR), DC-SIGN, and Dectin-2 on immune cells could modulate immunity to PA biofilms.

Methods: Binding assays and confocal microscopy were used to study CLR binding to PA biofilms and purified Psl. To investigate if biofilm exopolysaccharide composition influenced immune activation, huDCs (DC-SIGN+/MR+/Dectin-2+) were incubated with fixed biofilms (Psl+/Pel+, Psl+/Pel-, and Psl-/Pel+) alongside fixed non-biofilm forming bacteria (Psl-/Pel-), and cytokine production was quantified at 4 hours. Purified Psl and anti-CLR blocking antibodies were used to further investigate the interaction of biofilm-associated Psl and CLRs on huDCs.

Results: DC-SIGN, MR, and Dectin-2 bound to PA biofilms and purified Psl. Binding was calcium-dependent, competed with relevant CLR ligands, and was observed in biofilms formed by PAO1 and clinical isolates. Selective induction of IL-1β and IL-23 was observed in huDCs incubated with biofilms for 4 hours. Biofilms containing more Psl tended to induce less IL-23. Blocking two or more CLRs on huDC altered their cytokine response to biofilms.

Conclusion: We present the novel finding that Psl exopolysaccharide in PA biofilms can directly engage host CLRs and modulate immune activation. This furthers our understanding of PA pathogenesis and may have therapeutic implications.

OO7

Predicting antibiotic persistence and associated virulence in *Pseudomonas aeruginosa* biofilms from an *ex-vivo* perspective

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Background: Bacterial biofilms are known to have high antibiotic tolerance which directly affects clearance of bacterial infections in cystic fibrosis (CF) patients. Current antibiotic susceptibility testing methods are either based on planktonic cultures or do not reflect the complexity of *in vivo* biofilms, which affects treatment choice. This study aims to employ the *ex-vivo* pig lung model to study antibiotic persistence of *Pseudomonas aeruginosa* and associated virulence.

Methods: Sections of pig bronchiole were prepared and infected with clinical isolates of *P. aeruginosa* and incubated in artificial sputum media to form biofilms. Lung-associated biofilms were then challenged with antibiotics and their bacterial load quantified, and replicates were assayed for protease and pyocyanin production in different conditions. All isolates were tested for antibiotic susceptibility using standard MIC and MBEC methods.

Results: The results showed increased antibiotic tolerance of *P. aeruginosa* at >500-1000-fold MIC against meropenem, demonstrating a persistent phenotype in the biofilm model, all tested bacterial isolates showed a sensitive and/or intermediate phenotype by MIC and MBEC methods. The results also demonstrate an increased extracellular virulence production under antibiotic treatment compared to the untreated, which recapitulate the CF clinical manifestation. Interestingly, these extracellular virulence factors varied in the presence of different antibiotics, at different concentrations and in co-infection with *Stenotrophomonas maltophilia*.

Conclusion: We demonstrate a clinically realistic model that recapitulates CF through the determination of antibiotic resistance profile, further understanding of the effect of treatment on *P. aeruginosa* virulence and interspecies interaction with its associated virulence.

OO8

Understanding Phenotypic Diversity of *P. aeruginosa* in Chronic Diabetic Ulcers

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Understanding Phenotypic Diversity of *P. aeruginosa* in Chronic Diabetic Ulcers Chronic wounds (CW) are a common complication of diabetic ulcers (DUs), which are a major burden to health care systems worldwide and can result in lower limb amputation due to the intractability of the infection. In DUs there is a high probability of the infecting bacteria evolving considerable phenotypic and genetic diversity, as has previously been shown for chronic cystic fibrosis lung infections. However, it is not known whether this is also the case for DUs, and whether diversity impacts on virulence and antibiotic resistance. To study this, bacterial populations were isolated from different samples from patients with DUs. Phenotypic diversity was investigated in *P. aeruginosa* populations through the analysis of phenotypes traditionally associated with pathogenicity, and through whole genome sequencing.

Phenotypic variation in *P. aeruginosa* isolates taken from different patients was observed, but little variation within the same CW. Antibiotic resistance was found to increase during the course of infection, and it became apparent that *P. aeruginosa* colonisation in DUs is via a single strain per ulcer, and potentially per patient. In one patient, two distinct *P. aeruginosa* phenotypic profiles were found, so a detailed genomic analysis between isolates was carried out, including a full characterisation of the single nucleotide polymorphisms and a comparison of their transcriptomes using RNAseq. The results obtained suggest that the loss of flagellum may have facilitated evasion of the innate immune system, such that the blood isolates were able to go undetected and so spread systemically causing the rapid decline in the patient's health.

10th September

OO9

Air pollution differentially affects the *Staphylococcus aureus* oxidative stress response

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Particulate matter (PM) is a component of air pollution and is responsible for 1.5 million deaths associated with respiratory infections annually. Surprisingly, the impact of PM on bacterial behaviour is largely unknown. Our ground-breaking data showed that PM effects bacterial behaviour. Black carbon (BC), a major component of PM, altered biofilm composition and structure of *S. pneumoniae* and *S. aureus*, and increased dissemination of S. pneumoniae in *in-vivo* colonisation model[1]. However, the biological mechanisms responsible for these findings and the impact of PM on bacterial stress responses are unknown. To investigate the impact of PM on S. aureus further, the effects of BC and two sources of brake-wear PM on the S. aureus transcriptome were assessed. While the detrimental effects of PM on human health is well researched, the impact of non-exhaust PM specifically is lesser known; despite being a significant source of PM. Mixed brake dust" (BAD) was produced from the wear of brakes and "non-asbestos organic brake pad" (NAO) was produced from accumulation of brake pad wear only. Our transcriptional analysis for the first time shows that both exhaust and non-exhaust PM induces the S. aureus oxidative stress response. Interestingly the various sources of PM were found to elicit differential oxidative stress responses. Therefore our data demonstrates that these forms of PM are toxic to bacteria but S. aureus is able to counteract this toxicity. These findings may have significant value on understanding the complicated interactions between air pollutants, pathogenic bacteria, and the innate immune response.

[1]Hussey, S., Purves, J., Allcock, N., Fernandes, V., Monks, P., Ketley, J., Andrew, P. and Morrissey, J. (2017a). Air pollution alters *Staphylococcus aureus* and *Streptococcus pneumoniae* biofilms, antibiotic tolerance and colonisation. Environmental Microbiology.

OO10

An unusual feature in the subcellular landscape of long-term nitrogen starved *Escherichia coli*

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RNA binding proteins play diverse roles in the regulation of RNA metabolism. In many bacteria of diverse lineages, the RNA binding protein Hfq is a pleiotropic regulator of RNA metabolism and contributes to post-transcriptional regulation of gene expression by stabilising small non-coding RNAs and promoting their interactions with cognate mRNAs, ribosome biogenesis and indirectly on nucleoid conformation. Using single molecule photoactivated localisation microscopy analysis of individual Hfq molecules in live bacterial cells we now unravel an unusual feature of Hfq. We demonstrate that Hfq forms distinct foci in long-term nitrogen starved *Escherichia coli*. We describe the discovery and properties of the Hfq foci and propose that they represent subcellular structures in bacteria which are analogous to processing bodies (P-bodies) or stress granules found in stressed eukaryotic cells.

OO11

Path-seq identifies an essential mycolate remodeling program for mycobacterial host adaptation

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Background: Mycolic acids (MA) are distinct long-chain fatty acids found in the cell wall of mycobacteria, including the causative agent of tuberculosis (TB), *Mycobacterium tuberculosis* (Mtb). Understanding MA biosynthesis and regulation in Mtb and their role in the pathogenesis of active and latent infection is critical for the development of new vaccines and anti-TB drugs to fight the world's deadliest disease.

Methods: Path-Seq, a new method that we developed, was used to obtain global gene expression of both host and pathogen during infection. Among the up-regulated Mtb genes were desaturases involved in MA biosynthesis. Gene regulatory influence network (EGRIN) model of Mtb was used to identify the transcription factor (TF) regulating these desaturases. To confirm the desaturases repression, the viability of the inducible TF-overexpression strain was checked in solid media, and its lipid profile was analysed by thin layer chromatography.

Results: Using Path-seq, we observed significantly induced expression of MA biosynthesis genes, such as *desA1* and *desA2*, 24 h after Mtb infection of mice. Using multiple systems-level approaches, we identified MadR (Rv0472c) as *desA1* and *desA2* regulator. MadR-mediated repression led to the loss of mycobacterial viability and reduced MA biosynthesis.

Conclusions: Using Path-seq and regulatory network analyses we have discovered that MadR transcriptionally modulates two MA desaturases, DesA1/A2, to initially promote cell wall remodelling upon *in vitro* macrophage infection and, subsequently, reduce MA biosynthesis upon entering dormancy. As a result, we present MadR as a new and important antitubercular target.

OO12

A conserved protein, BcmA, mediates motility, biofilm formation, and host colonisation in Adherent Invasive *Escherichia coli*

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Adherent-Invasive *Escherichia coli* (AIEC) is a non-diarrhoeagenic intestinal *E. coli* pathotype associated with Crohn's Disease. AIEC pathogenesis is characterised by biofilm formation, adhesion to and invasion of intestinal epithelial cells, and intracellular replication within epithelial cells and macrophages. We have identified and characterised a protein in the prototypical AIEC strain LF82 which is required for efficient biofilm formation and dispersal – LF82_p314. LF82 Δ*LF82_314* have defective swimming and swarming motility, indicating LF82_p314 is important for flagellar-mediated motility, and thus surface colonisation and biofilm dispersal. Flagellar morphology and chemotaxis in liquid appear unaffected by deletion of *LF82_314*, suggesting LF82_p314 does not elicit an effect on flagella biogenesis or environmental sensing. Flagellar motility has been implicated in AIEC virulence, therefore we assessed the role of LF82_p314 in host colonisation using a *Caenorhabditis elegans* model. We found that LF82 Δ*LF82_314* have an impaired ability to colonise the *C. elegans* compared to wild-type LF82. Phylogenetic analysis showed that *LF82_314* is conserved in several major enterobacterial pathogens, and suggests the gene may have been acquired horizontally in several genera. Our data suggests LF82_p314 may be a novel component in the flagellar motility pathway and is a novel determinant of AIEC colonisation. Our findings have potential implications not only for the pathogenesis of Crohn's Disease, but also for the course of infection in several major bacterial pathogens. We propose a new designation for *LF82_314*, *b*iofilm *c*oupled to *m*otility *A*, or *bcmA*.

013

Discovery of compounds which reduce transmission of antimicrobial resistance plasmids

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Background: Genes encoding resistance to carbapenem and extended spectrum beta-lactam antibiotics are frequently located on plasmids. These plasmids can be transmitted between different bacteria. AMR plasmids can be maintained within bacterial populations over long periods of time, and transmission occurs on a global scale. Therefore, alternative approaches to identify compounds that prevent transmission of clinically important plasmids could contribute towards reducing the global burden of AMR.

Methods: We have engineered two globally successful plasmids carrying *bla*$_{\text{CTX-M-14}}$ and *bla*$_{\text{KPC}}$ to encode fluorescent proteins. The modified plasmids were inserted into *E. coli* or *K. pneumoniae,* allowing us to monitor plasmid transmission using confocal microscopy and flow cytometry. Using this system, we tested compound libraries for anti-plasmid activity. Potential hits were confirmed using dose response assays. The impact of hits upon bacterial growth was measured. Minimum inhibitory concentrations of top hits were determined.

Results: Our screen has identified compounds which show varying degrees of anti-plasmid activities. The top hits show potent and dose dependent reduction in plasmid transmission. Furthermore, they were able to reduce transmission at levels which did not impact bacterial growth, and are well below the minimum inhibitory concentration of the compounds.

Conclusions: Using our fluorescent transmission assay, we have identified compounds which reduce the transmission of "real-world" AMR plasmids amongst clinically relevant Gram-negative bacteria. Anti-plasmid approaches could be used in targeted areas to reduce the proportion of AMR bacteria, increasing the likelihood of bacteria being susceptible to existing antibiotics.

OO14

Hypervirulent, and MDR lineages of *klebsiella* replicate in macrophages *in vitro*, in murine organs, and in an *ex vivo* model of porcine liver and spleen co-perfusion

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Hypervirulent (HV) and multi-drug resistant (MDR) *Klebsiella pneumoniae* (*Kp*) represent a huge burden on human health. *Kp* are traditionally considered extracellular pathogens, but recent reports have indicated they can persist within macrophages. In this study, we characterised the growth and persistence of a panel of HV (serotype K1 and K2) and MDR (K107, and K17) *Kp* clinical isolates in a number of infection models.

In J774a macrophages, K1 isolates were highly resistant to phagocytosis, but displayed the greatest fraction of live intracellular organisms compared to serotypes K2, K17, and K107. All *Kp* strains were capable of replication within this macrophage type.

Following intravenous infection of mice, HV strains persisted in the blood, and had larger numbers of cultivable CFU in both spleen and liver compared to MDR strains. Microscopy analysis using whole tissue, multi-spectral imaging indicated (1) that infection is heavily bias towards CD169+ macrophages, and (2) that HV strains displayed larger macrophage loads compared to MDR strains. Confocal microscopy localised clusters of *Kp* intracellularly in macrophages.

Next, we developed a novel *ex vivo* co-perfusion of porcine spleen and liver which recapitulated physiological parameters. Following infection of this circuit, bacteria were rapidly cleared from the blood, but effectively replicated within the both splenic and liver tissue. Microscopy analysis localised these organisms – in parallel to the mice data – to macrophages.

In conclusion, we have demonstrated that diverse clinical isolates of Kp are able to persist and replicate within macrophages, and that this phenomenon may contribute to pathogenesis.

OO15

An intriguing link between membrane lipid remodelling and antimicrobial resistance in pathogen *Pseudomonas aeruginosa*

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Numerous bacterial phyla are able to remodel their membrane lipid composition in response to environmental stresses, in particular phosphorus deficiency. An intracellular phospholipase C, PlcP, cleaves the head group of membrane phospholipids, liberating diacylglycerol as a building block for non-phosphate lipid classes. Here we show that important opportunistic pathogen, *Pseudomonas aeruginosa* (*P. aeruginosa*), utilises the PlcP pathway, alongside glycosyltransferase enzymes to produce glycolipids in response to phosphorus stress. HPLC-MS (high performance liquid chromatography coupled to mass spectrometry) revealed the production of two glycolipids, monoglycosyl diacylglycerol (MGDG) and glucuronic acid diacylglycerol (GADG). Through *P. aeruginosa* mutant analysis and heterologous gene expression in *E. coli*, two glycosyltransferases, Agt1 and Agt2, located distinctly from one another, were found to be responsible for the production of MGDG and GADG glycolipids, respectively. *P. aeruginosa* is particularly problematic in terms of antimicrobial resistance as it has high natural resistance to many classes of antibiotics. Under phosphate stress, *P. aeruginosa* shows decreased susceptibility to cationic antimicrobial peptides (AMPs). Here we show that the ability to produce glycolipids in response to phosphate stress confers some protection to polymyxin B, a clinically used AMP. In particular, the inability to synthesise neutrally charged MGDG results in increased death for *P. aeruginosa* under low phosphate conditions, suggesting a protective role for this glycolipid in wild type conditions. Together this work characterises a pathway novel to *P. aeruginosa*, revealing the production of glycolipids in response to phosphate stress. Further, lipid remodelling impacts interaction with antimicrobial peptides in clinically relevant low phosphate conditions.

OO16

Mesoscopic Microbiology Exploring the inner workings of *E. coli* biofilms using the Mesolens

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Understanding the architecture of bacterial communities is crucial for developing novel methods of eradication and reducing their burden on public health. However, conventional imaging techniques are limited by sacrificing either the size of the imaging volume or the spatial resolution. Here we use the Mesolens, an optical microscope with a unique combination of a low magnification (x4) and a high numerical aperture (0.47) which can image volumes exceeding 100 mm^3 with lateral resolution of 700 nm and axial resolution of 7 μm. We report the formation of an intra-colony channel system which forms as an emergent property of colonial growth of *Escherichia coli* on a solid surface. We visualise the internal architecture of mature *E. coli* biofilms using widefield and confocal laser scanning mesoscopy and assess the functional role of intra-colony channels using fluorescent microspheres and nutrient sensing. Using an arabinose biosensor, we characterise the role which these structures play in nutrient uptake and delivery to the centre of mature *E. coli* biofilms. These findings illustrate a novel mechanism by which *E. coli* organise to form complex structures to promote their survival and could be exploited to develop novel methods for biofilm eradication.

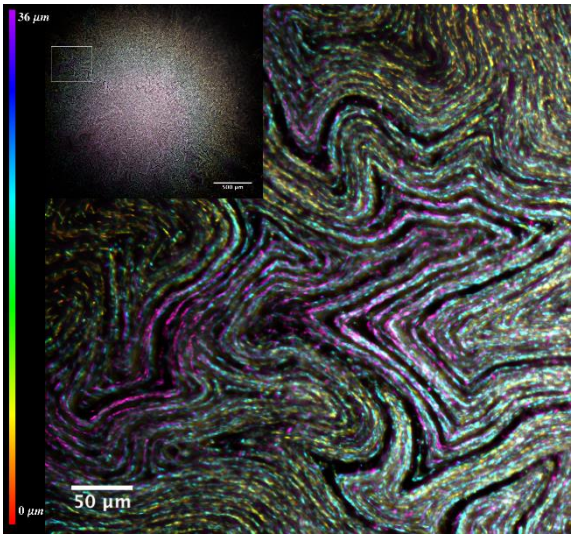

Figure 1: An *E. coli* macro-colony biofilm with a 3D network of intra-colony channels which facilitate the uptake of substances

Abstracts: Posters

M4_2019_P1

Prevention is the best cure – identifying the key properties of polymers that reduce dangerous biofilm formation

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Bacteria adhere to almost every surface and, in the context of healthcare, medical-device biofilm-centred infections pose an enormous threat, particularly from multi-antibiotic resistant superbugs. The fundamental problem lies in the fact that following an initial reversible surface-attachment, bacteria become irreversibly surface-attached, leading to the formation of antibiotic-tolerant/resistant biofilms that are almost impossible to eradicate.

Previously, using a developed high-throughput microarray screen with multi-antibiotic resistant pathogens (e.g. *Pseudomonas aeruginosa*), our team identified a class of polymers (RPs) that resist bacterial biofilm formation. The biological mechanism(s) by which the newly discovered resistant polymers (e.g. BACTIGON™ currently in clinical trials) prevent biofilm formation are still unknown.

In this study, through high-throughput single-cell tracking algorithms and microfluidics, we explored events during bacterial surface contamination. At the early stages, *P. aeruginosa* showed weaker surface adhesion strength, and moved faster and with shorter residence time on RPs than on pro-biofilm materials. We also observed that *P. aeruginosa* post division escape RPs with a higher frequency than when exposed to pro-biofilm surfaces.

In addition, we investigated the intracellular pathway used by *P. aeruginosa* to respond to surfaces. Our results suggest that bacteria actively "decide" whether to attach to surfaces and that the failure to form a biofilm on RPs is caused by the inability to switch from reversible to irreversible attachment.

This data provides insights into a new class of biofilm-resistant polymers that prevents bacterial irreversible attachment- taking us a significant step closer to discovering novel solutions for the prevention of bacterial infections.

M4_2019_P2

Characterization of the anti-oomycete activity of a rare sugar against *Phytophthora* spp.

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Members of the oomycete genus *Phytophthora* are among the most aggressive and widespread plant pathogens. These oomycetes are responsible for important diseases of many plant species worldwide and are the cause of significant crop losses that led to the increase of chemical pesticide input in agriculture in the last decades. However, the overuse of chemical pesticides produced many threats to human health and the environment. Therefore, natural compounds are urgently needed to further develop more sustainable strategies for the control of *Phytophthora* species. The aim of this study was to investigate the anti-oomycete activity and the mode of action of a rare sugar (SUG) against *Phytophthora infestans* and *P. cinnamomi* using biochemical and molecular approaches such as the assessment of oxygen consumption rate, the measurement of ATP turnover and quantitative PCR. Interestingly, SUG inhibited *P. infestans* growth by impairing mitochondrial respiration and causing oxidative stress with a consequent reduction of ATP synthesis and induction of the expression of stress-related genes. Conversely, SUG was not toxic against *P. cinnamomi* and none of the aforementioned alterations were observed. This research showed that SUG is a promising compound that might be developed as an innovative biopesticide for the sustainable control of *P. infestans*.

M4_2019_P3

Mercury Resistance Transposons: Multidrug Resistance Smugglers in Wastewater

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Since the first identification of a mercury resistance transposon in R100 isolated from *Shigella flexneri* in the late 1950's, the transposable element Tn*21* has since been identified in self transmissible plasmids and on the chromosome distributed across the globe in many *Enterobacteriaceae.* This element carries a class 1 integron which is located between the transposase genes and the mercury resistance operon and is separated by 25bp imperfect inverted repeats. The integron relies solely on the action of the transposon for the transmission of the resistance cassettes. Whilst this transposon is very well characterised, the integron is the most varied region as it may acquire and disinherit resistance cassettes freely. The contents of the integron can vary greatly due to environmental factors. In this study, *Eschericia coli* were isolated from multiple points in a waste water treatment system and screened for the presence of the Tn*21*. Approximately 10% of all *E. coli* isolates tested positive for the transposable element. Consequently these isolates were then screened for antibiotic sensitivity using disc diffusion assays. Of the isolates screened, 33% contained resistance to 2 or more classes of antibiotic. Two such isolates possessed the *Salmonella* genomic island in an IncF plasmid. One isolate later revealed through pulse field gel electrophoresis that it contained 2 plasmids between 120 and 150kb and a third plasmid circa. 45kb. It is unknown whether presence of antimicrobials or Hg2+ induce transposition of Tn21. Experiments are being conducted to test whether environmental factors or antimicrobial agents induce or enhance transposition.

M4_2019_P4

Can the microorganisms on fruit surface be the major potential source of attraction of *Drosophila suzukii*?

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Spotted wing drosophila *(Drosophila suzukii)*, is a major invasive pest of soft fruits in Europe and Americas. It is causing serious losses in the production of the most important soft fruits. Laboratory experiments were carried out to evaluate the behavioral responses of *D. suzukii* females to odors released by fresh and ripened fruits of non-crop hosts; wild mulberry (*Morus alba*), wild cherry (*Prunus avium*), and wild European blackberry (*Rubus fruticosus*). The behavioral response of *D. suzukii* naive mated females to fruit odors was investigated in two-choice bioassays using Y-tube olfactometer. The host samples were tested, separately in olfactometer, as non-sterilized and sterilized fruits. It was found for the first time that *D. suzukii* female flies were more attracted to non-sterilized fruits than sterilized ones. The highest attractiveness of fruits was recorded in wild European blackberry (*Rubus fruticosus*) which can be related to microorganisms associated with surfaces of fresh fruits. The results provide grounds for further investigations on microorganisms on fruit surface and development of a new trapping system. The traps baited with a new lure can contribute in setting up advanced eco-friendly control methods such as mass-trapping and attract & kill techniques.

M4_2019_P5

The plasticity and features of the transcriptional landscape of nutrient starved bacteria

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Bacteria in their natural environments seldom encounter conditions that support continuous growth. Bacterial pathogens often face complex, dynamic and nutritionally restrictive host microenvironments and local hypoxia that change over time. In addition, nutritional deprivation represents a primary line of host defence to starve invading bacterial pathogens and thereby restrict their growth. Further, when bacterial pathogens are excreted from an animal host, e.g. pathogenic *Escherichia coli*, they face limited nutrient availability in the non-host environment. Hence, many pathogenic bacteria spend the majority of their time starved of essential nutrients, including carbon, nitrogen and transitional metals. Therefore, to survive prolonged periods of nutrient starvation, bacterial pathogens have evolved adaptive strategies, which enable them to survive until conditions become favourable for growth. The *initial* response by which bacteria adapt to nutrient starvation manifests itself through large-scale reprogramming of the transcriptome. How the transcriptome temporally changes during *long term* nutrient starvation and how such changes enable bacterial survival, influence bacterial pathogenicity, and affect bacterial susceptibility to antibacterial stresses are not well understood. In this work, we used nitrogen starvation has a model stress to study how the transcriptional programme of *E. coli* changes during long term nitrogen starvation and reveal how the plasticity of the transcriptional landscape of nutrient starved bacteria affects the phenotypic properties of such bacterial populations.

M4_2019_P6

Metabolic profiling of volatile organic compounds produced by *Lysobacter capsici*: Towards biological control of plant pathogens

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The genus *Lysobacter* includes various biocontrol species that can produce a variety of secondary metabolites which exhibit activity against phytopathogens. Recently, it has been shown that volatile organic compounds (VOCs) produced by *L. capsici* DSM 19286 were capable of inhibiting the growth of *Phytophthora infestans in vitro,* after the strain had been grown in a protein rich medium [1]. Based on these findings, we have studied the inhibition activity of another biocontrol *L. capsici* strain against plant pathogens (e.g. *Rhizoctonia solani*). In parallel, GC-MS was combined with dynamic headspace (DHS) extraction and thermodesorption to investigate both type and relative amount of VOCs produced by the bacterium grown in various media. The chemical group of pyrazines was the most abundant in the volatile profile of the *L. capsici* strain. We additionally conducted experiments using a setup with Petri dishes having two compartments, where we measured the VOCs present in the compartment opposite of that in which the bacterium was grown. We confirmed the presence of the previously identified VOCs, suggesting that these compounds may contribute to the strain's bioactivity against the pathogens. Moreover, pure standard compounds of the identified VOCs did also lead to inhibition of the phytopathogens growth *in vitro* in a concentration dependent manner. Currently, we are studying further volatile components that presumably contribute to the strain's antifungal activity, with the aim to better understand the molecular mechanisms of VOC-mediated microbe-microbe communications and to add a stepping stone towards the development of novel biopesticides.

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M4_2019_P7

Interaction of bacteriophage with bacterial outer membrane vesicles:

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One of Gram negative organisms virulence factors are outer membrane vesicles (OMVs) that are produced during bacterial growth especially when faced with environmental stressors. OMVs are spherical microparticles and of similar composition as parent bacterium. OMVs were found to be a tool to inactivate bacteriophage upon interaction in order to protect bacterial parental cells. At the same time, OMVs are recognized by host immune system upon pathogen infection and participate in elicitation of host immune response. In this project, we aim to study the interaction between bacteriophages and OMVs, then to assess whether their interaction affects the inflammatory host response elicited by OMVs. Up to now, extraction of OMVs methods are being validated, isolation of novel phages has been successful and it has been shown that at least one of the isolated phages interacts productively with OMVs, and this interaction results in the inactivation of this phage *in vitro*. Further experiments are being carried out to investigate microbiological consequences of OMVs interaction with these phages. Furthermore, after confirming the interaction *in vitro*, this project will be intended to study immunological consequences of phage-OMVs complex.

M4_2019_P8

Characterising biofilm formation and AMR in *Pseudomonas aeruginosa* washing machine isolates

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Biofilms are communities of microorganisms that attach to various surfaces and are associated with infections in animals and plants. Our investigation is focussed on a current and growing concern: the distribution and formation of biofilms in washing machines. Many countries wash clothes at reduced temperatures around 30°C to 40°C degrees rather than at higher temperatures above 60°C that would kill the bacteria. Survival of the bacteria is associated with biofouling and malodour and have the potential to increase the risk of infection if human pathogens are present within the biofilms.

Pseudomonas aeruginosa is one of the predominant bacteria found in washing machines. *P. aeruginosa* is a ubiquitous environmental Gram-negative bacterium that is also an important opportunistic human pathogen with an intrinsic resistance to many antibiotics. In this work, we focus on the isolation and matrix-assisted laser desorption/ionization (MALDI) identification of *P. aeruginosa* from household washing machines. Confocal laser scanning microscopy revealed an increased propensity of this isolate to form biofilms compared to the laboratory strain PAO1. In addition, the washing machine isolate had a decreased susceptibility to benzalkonium chloride, a biocide used in washing detergents. Whole genome analysis was performed to identify different genomic features relevant to antimicrobial resistance (AMR) and biofilm formation. Furthermore, testing of different washing detergent formulations revealed a range of abilities to disrupt biofilm formation or kill *P. aeruginosa,* which will facilitate the development of more effective washing agents to limit the emergence of AMR within biofilms resident in domestic appliances.

M4_2019_P9

2-(2-Hydroxyphenyl)-thiazole-4-carbaldehyde (IQS) is a Byproduct of the Pyochelin Pathway and not a Pseudomonas aeruginosa Quorum Sensing Signaling Molecule

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Pseudomonas aeruginosa employs 3 well-studied quorum sensing (QS) systems – *las*, *rhl* and *pqs* that respectively depend on 3-oxo-C12-HSL, C4-HSL and PQS. In addition, a QS signalling molecule identified as 2-(2-hydroxyphenyl)-thiazole-4-carbaldehyde (IQS) was reported to modulate the QS network under phosphate limitation which favours *las*-independent activation of the *rhl* and *pqs* systems. Biosynthesis of IQS was suggested to depend on the *ambBCDE* gene cluster such that an *ambB* mutant exhibited reduced production of pyocyanin, elastase, PQS and C4-HSL which could all be restored by provision of exogenous IQS. However, IQS is chemically identical to aeruginaldehyde, a by-product of pyochelin biosynthesis whereas the *ambBCDE* gene cluster is known to direct synthesis of the antimetabolite L-2-amino-4-methoxy-*trans*-3-butenoic acid (AMB). To clarify the origin and contribution of IQS to the *P. aeruginosa* QS network, we synthesized IQS and a series of related compounds including aeruginoic acid, dihydroaeruginoic acid and aeruginol. In a *P. aeruginosa ambB* mutant neither the production of pyocyanin nor elastase nor expression of *pqsA*, *rhlI*, *lasI*, *phzA1* nor **phzA2** were influenced under phosphate limiting conditions by the provision of either IQS, an IQS analogue or pyochelin. LC-MS/MS showed that production of IQS by P. aeruginosa was not affected by mutation of the ambA or ambB genes but was abolished in a pyochelin biosynthesis (ΔpchAB) mutant. These data indicate that IQS does not modulate the QS network nor does it originate from the AMB pathway but is a by-product of the pyochelin biosynthesis pathway.

M4_2019_P10

The role of naturally occurring plasmids in the lifestyle choices of *P. fluorescens*

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Pseudomonas fluorescens is a growth promoting bacteria that colonises the rhizosphere of plants, including economically important crops such as wheat. *P. fluorescens* readily take up mobile genetic elements, such as conjugative plasmids. The presence of these plasmids has been shown to alter the transcriptome of their bacterial hosts. It has been observed that the presence these plasmids can lead to phenotypical changes, for example reduction in motility, which are known to be normally under the control of the Gac/Rsm regulatory system. The Gac/Rsm regulatory system is highly conserved within *Pseudomonas* species and is known to control the change between the acute and chronic lifestyle.

Recent work has discovered the presence of a small Rsm homologue, RsmQ, encoded on the pQBR103 plasmid. This has been shown to interfere with the native Gac/Rsm system. Using a combination of over-expression and phenotyping experiments we have found that RsmQ reduces motility and siderophore production whilst increasing biofilm formation, strongly suggesting that the plasmid based RsmQ protein is able to alter the host's transcriptome. To further investigate this, biochemical and biophysical techniques are being used to elucidate the mechanism by which this interference occurs. By understanding these mechanisms, we can better understand how transcriptional regulation occurs in all pseudomonads.

M4_2019_P11

Exploring the role of the iron sulfur cluster regulator IscR in *Yersinia* species.

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Yersinia pseudotuberculosis is an enteropathogen that is transmitted through contaminated food or water and results in self-limiting fever and gastroenteritis. *Y. pseudotuberculosis* is closely related to *Yersinia pestis,* the cause of bubonic, pneumonic and septicaemic plague. Despite causing vastly different diseases virulence is regulated in both species by several interrelated systems that include type three secretion, biofilm formation, motility, aggregation and quorum sensing. Recently a novel component of this network has emerged, the iron-sulfur cluster regulator, IscR. IscR is a transcription factor best understood for its role in regulating the formation of Fe-S cluster containing proteins in *E.coli*. It is now known that in *Y. pseudotuberculosis* IscR regulates type three secretion, a key virulence mechanism employed by pathogenic *Yersinia* spp. to inject effector proteins into host cells which have a range of effects including dampening the immune response and inducing apoptosis. Mutants were constructed in *Y. pseudotuberculosis* and *Y. pestis* in order to further investigate what role IscR plays in the regulation of virulence-related traits. As a virulence regulator IscR could be a future target for alternative antimicrobial therapies, a necessity given the threat of multidrug resistance and the classification of *Y. pestis* as a re-emerging pathogen.

M4_2019_P12

SNP Based Transmission Study of Badgers Infected with *Mycobacterium bovis* in the Edge Risk Area of England

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Mycobacterium bovis is the causative agent of bovine tuberculosis (bTB), one of the most costly and persistent agricultural infectious diseases still widespread in England today. However, not all parts of England are created equally with respect to the incidence of bTB and in 2012 the Department for the Environment, Food and Rural Affairs (DEFRA) divided the country into three risk areas; the high, low and edge risk areas.

The perpetuation of the bTB epidemic is blamed on the known wildlife host of *M. bovis*, the European badger (*Meles meles*). Despite this, no large scale studies examining both the prevalence and transmission patterns of *M. bovis* has been undertaken.

Here we describe the results of a major survey of 600 badgers from 6 counties within the edge risk area of England. *Mycobacterium* like colonies were isolated from over 80 indivduals of which, 53 were confirmed to be tuberculosis complex positive by PCR. These isolates were spoligotyped, VNTR typed and then subsequently whole-genome sequenced. We describe a SNP based transmission analysis of the sequenced isolates that provides a higher degree of resolution between the badgers compared to the described molecular typing methods.

M4_2019_P13

Flagellar motor in *Campylobacter jejuni* – microbiological and computational studies

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Campylobacter jejuni is a prominent food pathogen in developed countries and motility is important for its host-colonisation ability. It is a gram-negative bacterium with one flagella positioned in each pole, making it an interesting and suitable model organism for flagella assembly and placement research.

Previous research has uncovered an uncharacterized connection between FlhF, a regulatory factor involved in flagella assembly and FliF, one of the earliest assembling parts of the flagella biogenesis cascade. Occurrence of motile pseudorevertants in a non-motile Δ*flhF* strain has shown that mutations in FliF, a flagellar motor structural protein can circumvent need for the otherwise crucial expression factor FlhF. The mutations were concentrated in a region of the FliF that has not been identified as crucial for flagella biogenesis before. A combination of computational and experimental methods will be used to explore the mechanism of reversion.

Current work is focused on complementation studies with FliF in deletion strains to support previously performed phenotypic assays and transcriptome characterization of the revertant and Δ*flhF* strains In order to investigate the pseudorevertants from a structural point of view the in silico predicted protein structures of the wild type and revertant FliF proteins are examined. In addition the mechanism of reversion is further explored through phenotypic analysis of a previously uncharacterized pseudorevertant.

M4_2019_P14

Investigating the contribution of RND efflux pumps to antimicrobial resistance and virulence factor production

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Pseudomonas aeruginosa contains multiple resistance-nodulation-division (RND) efflux pumps which actively export a diverse range of substrates across the double membrane into the extracellular environment. These systems are associated with antimicrobial resistance, virulence and biofilm formation. This study aims to further understand this link, with a view to identifying ways to interfere with bacterial efflux as a novel antibacterial drug strategy.

A series of mutants were created using the suicide plasmid pEX18Gm; chromosomal deletions of *mexAB-oprM*, *mexCD-oprJ*, *mexEF-oprN* and *mexXY* were constructed both alone and in combination in *P. aeruginosa* PAO1 subline Lausanne (PAO1-L). Minimum Inhibitory Concentration (MIC) assays were performed to determine the susceptibility of each mutant to a range of antibiotics commonly used to treat *P. aeruginosa* infection. In line with previous studies, strains lacking *ΔmexAB-oprM* were more sensitive to β-lactams and strains lacking *ΔmexXY* showed increased susceptibility to aminoglycosides.

Virulence phenotypes including pyocyanin, pyoverdin, rhamnolipid and exoprotease production were assessed. Strains PAO1-L *ΔmexAB-oprM ΔmexCD-oprJ ΔmexEF-oprN* and PAO1-L *ΔmexAB-oprM ΔmexCD-oprJ ΔmexEF-oprN ΔmexXY* were significantly less virulent than the parent strain. Similar results were observed in motility assays. LC-MS analysis of extracellular quorum sensing signalling molecules reveals that these effects might be due to secondary effects on the PQS system.

M4_2019_P15

Control of type III-mediated virulence by cyclic-di-GMP

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Human and agricultural infections caused by bacterial pathogens are a major issue for the global population, acting as a threat to human health, food security, and the global economy. These problems add up to billions lost each year from both the healthcare sector and the agricultural sector.

Pseudomonas is a model organism used to study host-pathogen interactions. These bacteria carry out efficient host infection using a type III secretion system, which injects effector proteins and virulence factors into target cells, altering cellular defence mechanisms and impacting host tissues.

Previous work indicates that cyclic-di-GMP, a secondary signalling molecule, binds to an export ATPase complex (HrcN) at the base of the type III machinery. This work sets out to evaluate the role of this interaction and to identify its impact on system function and regulation.

To test the effect of loss of cyclic-di-GMP binding for HrcN function, mutant constructs with alterations to key predicted binding residues were developed for *in vivo* plant infection analysis with *Pseudomonas syringae* DC3000 and *in vitro* protein-based biochemical analyses."

M4_2019_P16

The effect of biocide exposure on the antibiotic tolerance and biofilm formation on *Staphylococcus aureus*

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The biocide triclosan is used extensively in both household and hospital settings, resulting in chronic exposure to the biocide in individuals that use triclosan-containing products. Triclosan is thought to induce antibiotic tolerance and alter biofilm formation, although the underlying mechanisms causing these changes are yet to be elucidated. If true, triclosan may contribute to antibiotic treatment failures, and therefore requires investigation. To determine how triclosan induces antibiotic tolerance, *S. aureus* was pre-treated with triclosan prior to treatment with ciprofloxacin, rifampicin, and vancomycin. Planktonic *S. aureus* cultures pre-treated with triclosan had 1,000 fold higher viable counts compared to non triclosan pre-treated cultures. Inspection of biofilms by live/dead staining found that triclosan pre-treatment protected *S. aureus* biofilms from treatment with otherwise lethal doses of ciprofloxacin, rifampicin, or vancomycin. Biofilms of mutant strains with a defective stringent response were not protected from antibiotic treatment, even in the presence of triclosan. Confocal laser scanning microscopy revealed that incubation of *S. aureus* with triclosan altered biofilm structure, resulting in increased proportions of polysaccharide in the biofilm matrix that could potentially mediate protection against antibiotics. Mutants deficient in *codY*, a gene encoding the CodY repressor that is closely associated with the stringent response, exhibited the same biofilm phenotype. This indicates that the stringent response mediates CodY derepression of polysaccharide biosynthetic genes, leading to excess polysaccharide production in triclosan exposed biofilms. We suggest that triclosan triggers multiple global regulatory systems in *S. aureus*, subsequently inducing tolerance to multiple antibiotic classes and altering biofilm structure.

M4_2019_P17

Silver acetate treatment of an *Acinetobacter baumannii* infection in the *Galleria mellonella* model

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Background: Due to the global threat of antimicrobial resistance, there is ongoing work into sourcing novel antimicrobials for a variety of applications. Silver acetate has the potential to be used in antimicrobial coatings of catheter devices. *Galleria mellonella* larvae are therefore used in this experiment to check the efficacy and safety of silver acetate as an antimicrobial.

Methods: In the first experiment, larvae are injected with varying concentrations of *Acinetobacter baumannii*, a multi-drug resistant pathogen, to find the concentration of cells that causes significant death (80%) over 96 hours. The next test injects the larvae with different concentrations of silver acetate to check the safety of the compounds to the host. To deduce the efficacy of silver acetate as an antimicrobial, the larvae are injected with *A. baumannii* and subsequently treated with silver acetate. After which, Galleria survival and bacterial load are measured to determine efficacy.

Results: The concentration of bacteria that caused 80% death of Galleria over 96 hours was 10^8 cfu/10µl. With regards to the safety of silver acetate, all concentrations up to 40 mg/kg appeared safe. A silver acetate dose of 10 mg/kg was used in the treatment study against 10^8 cfu/10µl *A. baumannii*. After infection with 10^8 cfu/10µl *A. baumannii*; silver acetate at 10 mg/kg reduced larval death by 46.67%. Each experiment was carried out with 10 larvae and was repeated in triplicate on separate occasions.

Conclusions: At low doses, silver acetate can significantly reduce the mortality associated with *A. baumannii* infection ($P = <0.0001$).

M4_2019_P18

Experimental evolution selects clinically relevant antibiotic resistance in biofilms but with collateral tradeoffs

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The widespread usage of antimicrobials in modern clinical, veterinary and industrial practices has selected for the emergence of antibiotic resistant bacteria, which are increasingly hard to treat with currently available antibiotics. Most bacteria in nature exist in aggregated communities known as biofilms, which are inherently highly tolerant to antibiotics. There is currently a limited understanding of how biofilms evolve in response to antimicrobial pressure. Here we used a biofilm evolution model as a tool to study the effects of antimicrobial exposure on biofilms compared to planktonic cultures. We showed that biofilms of the model food borne pathogen, *Salmonella* Typhimurium rapidly evolve in response to exposure to three clinically important antibiotics. Adaptation to antibiotic stress imposed a marked cost in biofilm formation, particularly evident for populations exposed to cefotaxime and azithromycin. By pairing the evolution model with wholegenome sequencing, we were able to identify and characterise two distinct mechanisms of resistance to cefotaxime and azithromycin. Among others, we identified novel substitutions within the multidrug efflux transporter, AcrB (R717L and Q176K) and validated their impact in drug export as well as changes in regulators of this efflux system. We showed that the model biofilm system selects clinically-important mechanisms of resistance and can be used to help predict how biofilms evolve under antimicrobial pressure.

M4_2019_P19

"Better eight than never: The eighth RND efflux pump of *Escherichia coli*"

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It is well characterised that *Escherichia coli* has seven RND efflux pumps within its genome (AcrB, AcrD, AcrF, MdtB, MdtC, MdtF and CusA). However, we have now shown that the EefABC efflux pump, which was previously characterised in *Klebsiella aerogenes* (formerly known as *Enterobacter aerogenes*), is present in *E. coli* isolates from 13 different sequence types including many that cause invasive disease. Bioinformatic analysis of the pangenome of *E. coli,* comprising of 11,762 genomes, was performed to find the prevalence and percentage identity of the EefABC efflux pump. This pump is present in more than half of the *E. coli* sequence types analysed, furthermore the pump genes are almost entirely conserved. The sequence of this pump was also compared to other RND efflux pumps in *E. coli* and was found to be most similar to AcrAB and subsequently modelled against it. EefABC was cloned into a mutant *E. coli* deficient of main RND efflux pump AcrAB and the phenotypic role of this pump has also been evaluated. This study shows an additional efflux pump is present across clinically relevant *E. coli* sequence types and is highly conserved suggesting an important biological role.

M4_2019_P20

Chlorpromazine as a competitive substrate of the multidrug efflux pump AcrAB-TolC.

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Efflux pumps are an important mechanism conferring intrinsic and evolved multidrug resistance (MDR) in Gram-negative bacteria, recognising toxic substances and extruding them to the external environment before they interact with their intracellular targets. Inhibition of efflux is an important strategy to restore the antibacterial activity of antibiotic agents. Chlorpromazine has previously been shown to behave as an efflux inhibitor, increasing the intracellular concentration of AcrAB-TolC substrates. However, its mode of action is poorly understood. Understanding the mode of action of chlorpromazine as an antibiotic adjuvant is essential to facilitate the rational design of phenothiazine derivatives, or novel agents, active against MDR bacteria. The aim of this study was to determine whether chlorpromazine interacts with AcrB.

Chlorpromazine potentiated the activities of AcrB pump substrates against *S.* Typhimurium and *E. coli* and increased the intracellular accumulation of norfloxacin and decreased the efflux of ethidium bromide. Molecular modelling studies with AcrB from *S.* Typhimurium showed that chlorpromazine binds within the distal binding pocket at the hydrophobic trap of AcrB; a known efflux inhibitor binding site. These simulations show chlorpromazine is able to interact with residues of AcrB that are important for substrate binding and thereby may outcompete binding by norfloxacin and ethidium bromide. Additionally, exposure of *S.* Typhimurium, containing a non-functional AcrB (D408A), to chlorpromazine gave rise to mutants containing wild type AcrB. This provides further evidence to support this drug being a substrate of AcrB.

M4_2019_P21

The importance of Efflux is Growth Phase Dependent

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Gram-negative infections are difficult to treat due to an impermeable outer membrane and multiple efflux pumps maintaining low antibiotic accumulation within cells. The RND efflux pump, AcrAB-TolC is required for surviving antibiotic treatment in exponential phase but its stationary phase role is unknown.

Using flow cytometry, we developed a method to measure ethidium bromide (EtBr) accumulation in single cells. *Salmonella* Typhimurium cultures were grown in drug-free LB and samples taken hourly; EtBr dye was added and the level of accumulation measured after 10 minutes.

In WT *Salmonella*, EtBr accumulation was low in all samples regardless of which timepoint the samples were taken. In Δ*acrB*, samples taken during exponential phase had high EtBr accumulation, but surprisingly, this dropped to WT levels during stationary phase. This suggests efflux is most important in exponential growth.

This pattern could be explained by other efflux pumps becoming upregulated in stationary phase to compensate for AcrAB-TolC loss. Here, we show that strains lacking TolC, and the periplasmic adaptor proteins, have the same accumulation pattern as Δ*acrB*, and addition of an efflux inhibitor in WT strains produces a similar pattern. Using GFP transcriptional reporters, we show that transcription of the 4 other RND pumps does not increase in Δ*acrB* strains during stationary phase, so is not responsible for the phenotype seen.

Efflux is an attractive target for overcoming antimicrobial resistance, but in order to use inhibitors, it is fundamental to understand efflux and other factors involved in maintaining low antibiotic accumulation in different growth states.

M4_2019_P22

Sensitising *Pseudomonas aeruginosa* biofilms to antibiotics and reducing virulence through novel target inhibition.

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Background: *Pseudomonas aeruginosa* represents one of the top priority pathogens according to the WHO due to its high level of antimicrobial resistance. This organism controls the production of virulence traits at the bacterial population level through quorum sensing (QS). *P. aeruginosa* has several QS systems, one of which, the Pseudomonas Quinolone System (*pqs*), uses 2-alkyl-quinolones (AQ) as signal molecules. The *pqs* system plays a key role in controlling the production of virulence factors and biofilm formation representing a key drug target for virulence attenuation.

Hypothesis and aims: To identify molecules that are able to inhibit the interaction of PQS with PqsR leading attenuation the virulence of this organism, sensitising biofilms to the action of antibiotics.

Methodology: *In silico* screening was employed to identify ligands that inhibit the interaction of PQS with PqsR. This was then followed by validation using a bioreporter assay. The identified hits underwent a structure activity relationship to enhance their affinity for PqsR and improve their physiochemical properties. The antagonists were also subjected to a series of phenotypic analyses using *P. aeruginosa* cultures including pyocyanin, AQ signal quantitation and biofilm inhibition assays.

Results: We have identified a highly potent PqsR inhibitor (SEN089) which has significantly reduced pyocyanin expression and production of AQ signals. Moreover, SEN089 sensitised biofilm to tobramycin treatment with almost complete eradication within 6 h.

Conclusion: SEN089 represents promising novel anti-virulence compound against *P.aeruginosa* infections as it sensitises biofilm to antibiotic treatment.

M4_2019_P23

Molecular approaches to understand the effect of acetic acid in uropathogenic *E. coli*

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Acetic acid has long been known for its antibacterial activity. We are using TraDIS to investigate the molecular mechanisms by which acetic acid acts as an antibacterial agent.

To do this, we grew a high-density transposon library in uropathogenic *E. coli* EO499 serotype 131 in M9 media at pH 7 and pH 5.5 with acetic acid concentrations of 40 mM and 4 mM, respectively, or without added acetic acid. Sequencing libraries were generated from total bacterial populations after growth, and sequenced using a transposon-specific primer to generate positions and frequencies for each transposon. By comparing numbers of reads before and after the stress, we identified candidate genes where transposon inserts led to a decrease of fitness under acetic acid stress. Eight of these were chosen for further study: *nuoM, nuoG, sucA, sthA, pitA, apaH, rssB* and *ytfP.*

Because of the difficulties of constructing gene deletions in the uropathogenic strain for validating the TraDIS results, we tested the relative fitness of the corresponding gene deletion mutants from the Keio library (in strain BW25113), with the growth conditions used for EO499. Interestingly, only a few knockouts showed a reduction in relative fitness in time course competitions at pH 5.5 with acetic acid. This may due to the differences between strains used in TraDIS and competition. To overcome this issue, we have also isolated transposon mutants from *E. coli* EO499 transposon library for the determination of relative fitness. The results will be presented.

M4_2019_P24

The Metabolic Balance Between PQS-Dependent Quorum Sensing and Anthranilate Degradation is Shifted Upon Deletion of the Twin-Arginine Transport System

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The twin-arginine transport (TAT) system moves fully-folded and multimeric proteins from the cytosol to the periplasm. There are currently over 30 identified substrates in *Pseudomonas aeruginosa* and many of these are virulence factors. Tat mutants are avirulent and form aminoglycoside-sensitive biofilms with defective architecture and reduced extracellular DNA. Deletion of the *tat* genes in *P. aeruginosa* correlates with dysregulation of *Pseudomonas* quinolone signal (PQS)-dependent quorum sensing, a key chemical messaging system employed by *P. aeruginosa* to coordinate virulence and biofilm formation. Transcriptome analysis revealed upregulation of genes involved in anthranilate degradation in the tat mutant. As anthranilate can either be degraded and fed into the TCA cycle for energy production or used by PqsA for PQS biosynthesis, the relationship between these two pathways was investigated further. MiniCTX-*lux* transcriptional reporters were constructed to use bioluminescence as a measure of gene expression. The promoter regions of the first two operons of the anthranilate degradation pathway *antA* and *catB* and their respective regulators *antR* and *catR* were fused upstream of the *luxCDABE* operon on a miniCTX-*lux* vector and inserted into the bacterial chromosome. Expression of the genes in both a wild-type PAO1 strain, a *tatA* mutant strain, and a *pqsA* mutant strain were measured. The results show that both mutation of *tatA* and deletion of *pqsA* increase expression from the *antA, antR,* and *catB* promoters. Further work is underway to determine the reasons behind the observed metabolic shift away from PQS biosynthesis and towards anthranilate degradation.

M4_2019_P25

MICROBIAL ELECTROSYNTHESIS FOR DIRECT CONVERSION OF WASTE GASES INTO CHEMICALS

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The market for biofuels and microbially derived chemicals (bio-commodities) is experiencing a steady growth, as the microbial route for production presents many advantages in term of eco-sustainability and recycling of waste sources. However, microorganisms must rely on expensive energy-rich feedstocks for this process. To overcome this limitation, bioelectrochemical systems (BES) are emerging as an alternative for converting organic and inorganic waste into bio-commodities. The aim of this project is to engineer a flexible microbial electrosynthesis (MES) system to enable efficient and scalable production of added value chemicals from CO_2, using a recombinant strain of *Cupriavidus necator H16* (*C. necator*). As *C. necator* lacks the ability to efficiently accept electrons from an external source at a fast-enough rate and couple them to energy conservation, an exogenous electron conduit derived from *Shewanella oneidensis MR-1* has been engineered into the strain. Additionally, a novel protein construct designed to covalently attach to functionalized electrodes is expressed on the outer membrane. This system enhances the formation kinetics and stability of the biofilm, enabling the customization of spatial coverage to improve electron transfer. Moreover, in order to have flexibility in manipulating the conditions of the process (e.g. different electrode materials and/or topologies, growth conditions, etc.), a custom modular MES device will be realized for the specific target product. A significant part of the project will involve the design and development of the MES cell, manipulation, and optimization of the genetic pathways of *C. necator* to achieve a sustainable process with an improved product yield.

M4_2019_P26

Taming *Cupriavidus metallidurans* CH34 for recovery of hydrocarbon and heavy metal polluted wastewaters in Microbial Fuel Cells

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Treatment of wastewaters is an energetically demanding process, which has been estimated to consume up to 1.5% of the total electricity production in the USA. In the last two decades, Microbial Fuel Cells (MFC) rose as a promising technology for recovery of contaminated wastewaters with simultaneous production of electricity, but such systems are not employed in large scale yet due to low current density being produced. *Cupriavidus metallidurans* CH34 is an electroactive, facultative anaerobe, chemolitoautotrophic bacteria which has been recently found to form biofilms on gold surfaces. Its capability to withstand μM concentrations of heavy metals and degrade recalcitrant toxic compounds such as aromatic hydrocarbons, make it an ideal candidate for recovery of industrial wastewaters in MFC setups. We aim to engineer *Cupriavidus metallidurans* CH34 with autotransporter mediated surface expression of a SNAP tag which will promotes enhanced biofilm formation on the anodic surface functionalised with Single Assembled Monolayer endowed of O^6-Benzylguanine. Nevertheless, *C.metallidurans* CH34 is endowed with a large array of mobile elements and transporters coping with toxic metabolites such as antibiotics, which make expression of heterologous proteins and manipulation of its genome arduous challenges. Therefore we further want to develop an auxotroph based plasmid addiction system for stable expression of proteins as well as a *C.metallidurans* CH34 dedicated CRISPR tool for efficient engineering of genomic DNA.

M4_2019_P27

Antibody Responses to *Mycobacterium tuberculosis*

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Antibody responses against *Mycobacterium tuberculosis* (*M.tb*) are of increasing interest. Despite this, we have limited understanding of which antigens are recognised during active TB (ATB) disease or latent TB infection (LTBI), the pattern of antibody isotypes induced to mycobacterial antigens and the influence of treatment on these responses. This study systematically characterises antibody responses to *M.tb* and 7 proteinaceous and non-proteinaceous antigens in patients with ATB and LTBI and profiles the dynamic antibody changes in response to treatment.

Total IgG, IgG1-4 and IgA antibody responses of ATB and LTBI patients, against 7 and *M.tb* H37Rv whole-cell, were measured by ELISA. Serum samples were from 18 ATB; 20 LTBI and 12 healthy controls.

The dominant trends observed included higher pre-treatment antibody titres to *M.tb* antigens in the ATB group compared with the LTBI group. In addition, the antibody responses amongst the ATB group usually fell after treatment to similar levels as healthy controls. There were no clear trends in the LTBI group responses before treatment, often with little difference in response compared with healthy controls. Interestingly, the IgG3 response to *M.tb* antigens appeared to be particularly prominent amongst TB patients and perhaps is a dominant subclass.

These findings suggest that exposure to high levels of *M.tb* antigens during ATB may influence the magnitude of specific antibody responses, but that distinct differences exist between individuals with ATB and LTBI. Further investigation of human humoral responses to *M.tb* may provide new diagnostic, prognostic and therapeutic tools and provide insight into the host-pathogen interplay.

M4_2019_P28

The question would be: What mastery can we offer over the fruit damaging pest species, *Drosophila suzukii*?

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In 2008, a rapid invasion of the soft fruit pest species, *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) occurred across Europe and the Americas. Consequentially, *D. suzukii* is currently the number one pest of valuable horticultural crops, attacking soft fruits and wine grapes, and causing millions of dollars of damage annually. This small fruit fly, a sister species of *D. melanogaster* and *D. similans*, is able to detect fruit odours and penetrate ripening fruit and we expect that investigation of the olfactory system of *D. suzukii* will develop our understanding of the behavior and physiology of these insects and allow us to develop effective pest control solutions. Here, we use microbiological, chemical, electrophysiological, laboratory bioassays and open field studies in our investigations.

Sustainable approaches to limit the spread of and damage caused by *D. suzukii* requires effective lures. We are in the process of developing innovative and effective lures that will eventually be implemented in a selective trapping system for controlling SWD in Integrated Pest Management (IPM) programs in open fields. We developed Droskidrink as a commercial product for catching *D. suzukii*. We demonstrated that adding lactic acid bacteria to Droskidrink in the first week after fermentation improves attractiveness to SWD. We investigated key odourant cues for SWD, emitted from the wine-vinegar-lactic acid bacteria fermentation process. We are investigating odourant receptors of the sister species *D. similans,* and the need to reduce by-catch of non-target species. Ultimately, we hope to use this knowledge to develop a selective trapping system for SWD.

M4_2019_P29

The impact of a horizontally acquired virulence plasmid on *Bacillus cereus* G9241, the causative agent of an anthrax-like illness.

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Bacillus cereus, *Bacillus anthracis* and *Bacillus thuringiensis*, are Gram-positive, spore- forming bacteria and principle members of the so-called *Bacillus cereus* sensu lato complex. The species are highly similar at a chromosomal level, but are phenotypically diverse due to the presence of different plasmids. The anthrax pathogen, *B. anthracis* contains two virulence plasmids; pXO1, carrying the toxin gene, and pXO2 carrying the genes required for capsule production. Both plasmids are required to allow *B. anthracis* to act as a highly virulent mammalian pathogen. As well as encoding toxins, the pXO1 plasmid encodes *atxA*, a transcriptional regulator that is able to control gene expression from both the plasmid and chromosome. It is proposed that AtxA is incompatible with the chromosomally encoded global regulator PlcR and co-expression has been seen to impair sporulation. As a result this has led to the genetic inactivation of *plcR* in all *B. anthracis* isolates and driven the evolution of high mammalian virulence. Interestingly there are several *B. cereus* isolates that possess a pXO1-like plasmid, called pBCXO1, which are capable of inducing an anthrax like illness. Importantly, genome sequencing of one such strain, *B. cereus* G9241, revealed intact copies of both *atxA* and *plcR* genes. To understand the impact of pBCXO1 on the biology and life style of *B. cereus* G9241, a pBCXO1-cured strain has been used to study the influence of the virulence plasmid on the transcriptome of *B. cereus* G9241.

M4_2019_P30

The variation of RND efflux pumps in *E. coli*

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Bacterial efflux is associated with an array of phenotypes including antimicrobial resistance, virulence, and biofilm production. One of the best characterised efflux pumps is AcrAB-TolC which belongs to the resistance-nodulation-division family of pumps. This pump is able to transport a diverse range of molecules, including clinically relevant drugs. Furthermore, overexpression of AcrAB-TolC is associated with multidrug resistance, yet variation within RND pumps is not yet fully understood.

Pangenomes of 20 medically relevant sequence types (ST) of *E. coli* were assembled from over 19000 annotated genome assemblies. To investigate the conservation of 14 RND efflux genes, the available sequences from *E. coli* str. K-12 substr. MG1655 (NC_000913.3) were aligned against each pangenome.

The extent of gene conservation was found to be dependent on both gene and ST group. AcrA*,* AcrB and TolC are known to play an important biological role in *E. coli* and in this study were found to be highly conserved across all 20 ST. Most ST had only a single variant (identity ≥ 95%) for each component of the tripartite pump. However, other RND efflux systems were far more varied. For example, in *E. coli* ST10 and ST131, two ST associated with MDR plasmid carriage, high levels of variation were seen in the *cusABC* and *mdtABC* systems. Suggesting that at the pangenome level, *acrA, acrB* and *tolC* are highly conserved, while other efflux pump systems may be under varying degrees of selection pressure depending on *E. coli* lineages*.*

M4_2019_P31

The role of air pollution on the decline of essential insect pollinators

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The role of air pollution on the decline of essential insect pollinators

Bumblebees play a major role in global pollination. Consequently, their health is of high importance to our food security. Recent bumblebee declines are proposed to be due to a combination of habitat loss, pesticides and disease. However, the key reasons for the decline of these essential pollinators have not been fully determined.

Air pollution is a major global problem, as atmospheric pollutants cross national boundaries, settling on plants, soil, and water courses. Bees are exposed to airborne particulate matter (PM), with particulates associated with the wings, head and legs of honey bees. The bees take the particulates back to colonies/hives exposing the colony to the particulates via consumption and inhalation. Consequently, there is a strong possibility that PM is related to the decline in bees.

An important factor for bee health that contributes to population survival, is the gut microbiome composition. The bee gut microbiome is functionally comparable to the human gut microbiome as they both provide protection from pathogens, are specific to the host and help break down food. Without a balanced gut microbiome, the health of the bee is threatened through increased infection and mortality.

Previous published data shows that air pollution has an impact on bacteria. Therefore, our hypothesis is exposure to air pollution causes an imbalance in the bee gut microbiome. To test this, we exposed bees to black carbon, a major component of PM, and assessed the effects on bee behaviour and microbiome composition.

The bee gut microbiome is relatively simple, being dominated by 8 core bacterial species providing a convenient study system. Bees treated with BC showed a significant reduction in viable bacterial cells in their faecal community. This supports the hypothesis that air pollution can cause an imbalance in the bee gut microbiome, thus adversely influencing bee health and pollinator populations.

M4_2019_P32

Sheep feet as a vehicle to transmit antimicrobial resistant bacteria in the environment

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The overuse of antibiotics in both human and animal medicine has intensified selective pressure on bacteria, promoting multiple mechanisms of resistance, spreading rapidly through horizontal gene transfer. Antimicrobial resistant bacteria, transferred from animals to humans, impact on future food security and human health. The UK sheep industry represents the largest in Europe and tetracycline is the most common antibiotic used to treat infectious diseases such as footrot.

E. coli isolated in the presence and absence of tetracycline from ovine interdigital skin swabs were whole genome sequenced and the antibiotic sensitivity was tested by disk diffusion assays. Antibiotics of veterinary importance (spectinomycin, neomycin), critically important (fluoroquinolone & carbapenem) and highly important to human health (2nd generation cephalosporin) based on the WHO (World Health Organisation) classification were tested.

All of the *E. coli* isolates were multidrug resistant (3-4 classes, including sulphonamides and aminoglycosides) forming 11 resistance profiles, with two dominant ones: Profile 1 with resistance to tetracycline, streptomycin, spectinomycin and suplphatriad; Profile 2 with resistance to tetracycline, streptomycin, spectinomycin, sulphatriad and intermediate level for imipenem.

Although isolates found on the ovine interdigital skin were not resistant to antibiotics important to human health, they are multi-drug resistant to antibiotics used in the sheep industry. In particular, resistance to spectinomycin is of concern as it is used to treat neonatal lambs with colibacillosis (watery mouth). This study highlights the importance of

responsible use of antibiotics to slow the spread of resistance and to maintain effective treatment.

M4_2019_P33

Mycobacterial phosphatase PstP regulates global serine threonine phosphorylation and cell division

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Mycobacterium tuberculosis PstP is a conserved serine threonine protein phosphatase encoded near protein kinase B and cell division genes. In many Actinobacteria, PstP is the sole phosphatase to counter the activity of multiple kinases. We constructed a conditional mutant of the *pstP* operon in *M. smegmatis* to investigate its function. The resulting cell wall defects and differential phosphorylation of PknB and CwlM during knockdown suggest a specific role in regulating PknB and cell division, while hyperphosphorylation of multiple proteins, in particular phosphothreonine in membrane proteins, suggests PstP may also act as a general negative regulator of phosphorylation.

M4_2019_P34 Withdrawn

M4_2019_P35

Lipid remodeling: Does it play a role in antibiotic resistance in *Burkholderia cenocepacia*?

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The opportunistic pathogen *Burkholderia cenocepacia* mainly infects patients with cystic fibrosis and has high intrinsic resistance to a wide range of antibiotics. We have begun to investigate whether membrane lipid remodeling could be a previously overlooked mechanism of antibiotic resistance, specifically under phosphate limited growth conditions. Lipid remodeling has been well studied in an environmental context and is mediated by a phospholipase encoded by the *plcP* gene. The PlcP enzyme has been shown to degrade zwitterionic phospholipids during phosphate stress, which liberates phosphorus for critical cellular processes. This enzymatic reaction also generates diacylglycerol, which can be used to synthesize phosphorus-free membrane lipids, such as glycolipids. Here, wild-type *B. cenocepacia* was transferred to a phosphate-deplete medium for 24 hours and the membrane lipid composition analyzed using LC-MS/MS. Within 8 hours of encountering phosphate stress, the membrane lipid landscape changed with two glycolipids, monogalactosyl diacylglycerol and glucuronic acid diacylglycerol, being detected. These glycolipids have been identified as monogalactosyl diacylglycerol and glucuronic acid diacylglycerol. We have also constructed a *plcP* knockout mutant and are currently analyzing the role of *plcP* in the production of both of these glycolipids and whether these glycolipids are important for intrinsic resistance to specific antibiotics and ultimately pathogenesis.

M4_2019_P36

Defining the RND-binding residues of AcrA

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Active efflux, driven by efflux pumps, is one of the main mechanisms of antibiotic resistance. In particular, the resistance-nodulation-division (RND) family of efflux pumps confer clinically relevant antibiotic resistance in Gram-negative bacteria, such as *Salmonella enterica*. RND pumps, including AcrB, are organized as tri-partite systems, consisting of an inner membrane RND pump, a periplasmic adaptor protein (PAP) and an outer membrane channel. Previously, inactivation of the PAPs AcrA and AcrE in *S. enterica* has been shown to significantly increase susceptibility to antimicrobials and reduce virulence. Therefore, PAPs are seen as attractive targets for the development of efflux pump inhibitors. However, the role of PAPs in the assembly of tri-partite pumps and the residues involved in PAP-RND pump binding is poorly understood. In this study, the effect of point mutations in the predicted binding sites in AcrA on efflux activity was investigated through ethidium bromide efflux assays and MIC testing. Several point mutations were found to significantly impair efflux activity and increase susceptibility to antibiotics, suggesting an important role for G58, T270, T271, G272, S273, F292, R294 and G363 of AcrA in RND pump binding. The role of these residues in RND binding was further supported by molecular visualisation work using PyMOL. These residues could be important targets for the design and development of PAP inhibitors.

M4_2019_P37

Copper hyper-resistance in *Staphylococcus aureus USA300*

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Copper is an essential metal in eukaryotes and prokaryotes, however excess levels are toxic. Bacteria have developed mechanisms to counteract these toxic effects. Significantly, copper has been shown to be important in host innate immunity as an antibacterial mechanism against invading pathogens, via active transport of copper into the phagosome.

Worryingly, there has been a global emergence of *S. aureus* strains with increased antibiotic resistance (e.g community-acquired methicillin resistant *S. aureus* (CA-MRSA)), which unlike typical *S. aureus,* can infect healthy humans with no previous exposure to healthcare situations. These isolates show increased resistance to innate immunity and reduced clearance from healthy airways compared to other *S. aureus* clinical isolates.

Recently, we identified a novel horizontally transferred copper resistance locus in CA-MRSA which is in addition to the core copper homeostasis operon (*copAZ)* found in all *S. aureus*. This locus is not present in established *S. aureus* human lineages encodes a P_{1B-3}-type ATPase copper transporter and a lipoprotein (CopL) of unknown function. Our data show that the operon confers resistance to extremely high concentrations of copper compared to other *S. aureus* and, notably, were found to be important for survival within intracellular macrophages.

The recent evolution and success of USA300 may be due to possession of this additional copper resistance locus, enhancing bacterial fitness through increased resistance to copper-dependent bactericidal innate immunity. The research presented here will outline new insights into the currently unknown function of CopL, further establishing a role for this lipoprotein in *S. aureus* USA300 macrophage survival and copper hyper-resistance.

M4_2019_P38

Air pollution induces an adaptive response in Staphylococcus aureus and alters host-pathogen interaction.

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Air pollution is a major global health problem, with around 91% of the world's population living in areas that exceed the WHO air pollution guidelines. This complex mix of pollutants almost always includes particulate matter (PM), and this has the greatest impact on human health. PM exposure contributes to a range of diseases such as COPD, heart disease and respiratory infections. Our recent publication was the first to document that as well as damaging the host, PM has a direct impact on bacteria that can cause respiratory infections. We showed that Black Carbon (BC) exposure results in species-specific alterations in biofilm structure in both *Streptococcus pneumoniae* and *Staphylococcus aureus*, altered biofilm protectivity against antibiotic exposure, and *S. pneumoniae* bacterial colonisation *in vivo*.

Following on from this ground-breaking work, our current data show that the bacterial response to BC occurs at the genetic level, altering the transcription of key genes involved in biofilm formation, colonisation and virulence. Bacterial adhesion to and invasion of human epithelial cells is significantly increased when *S. aureus* are pre-exposed to BC prior to infection compared to naïve *S. aureus* cells. In a murine respiratory colonisation model, both *S. aureus* co-infected alongside BC, and crucially *S. aureus* pre-exposed to BC, show increased colonisation of the nasopharynx and lungs. These data suggest that the bacteria are responding and adapting to exposure to air pollution, and this has an impact on how the bacteria infect the host.

M4_2019_P39

Comparison of type 5d autotransporter phospholipase activities suggests a role in intracellular pathogenic lifestyle

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Background: Type V secretion systems (T5SSs) or autotransporters as well as bacterial phospholipases are known virulence factors in many pathogenic bacteria. The patatin-like protein D (PlpD) of *Pseudomonas aeruginosa* is the prototype of the subclass T5dSS, which secretes a lipolytic passenger that forms extracellular homodimers. We aimed to characterize the enzymatic activity, substrate specificity and multimerization status of homologues of PlpD from the pathogens *Aeromonas hydrophila*, *Burkholderia pseudomallei*, *Ralstonia solanacearum* and *Vibrio cholerae*.

Methods: The lipase domains of T5dSSs were produced recombinantly. A continuous fluorimetric assay was used to measure lipase activity of the target proteins. Substrate specificity was assayed using lipid strips, and oligomer formation was determined by size-exclusion chromatography and crosslinking.

Results: All studied phospholipases were active over a broad temperature and pH range, displayed phospholipase A1 (PLA1) activity and high-affinity binding to phosphatidylserine. Phospholipases from *A. hydrophila* and *B. pseudomallei* also showed PLA2 activity. Self-associated multimer formation is a conserved feature of T5dSS passengers. Yet, homodimer formation is not essential for enzymatic activity but rather for protein stability.

Conclusion: The exact role that T5dSSs play during infection has yet to be determined. However, the fact that several of the characteristics tested in this work are conserved across a wide range of pathogens and that there are distinctive correlations between enzymatic activity as well as substrate specificity and intra- and extracellular lifestyle suggests a role for T5dSS phospholipases in bacterial fitness during infection.

M4_2019_P40 (No flash)

Characterisation of recent clinical *Salmonella* Dublin isolates from bovine abortions

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Salmonella enterica serovar Dublin is one of the most common bacterial causes of abortion in cattle in the UK. Despite its prevalence, little is understood about the progression of the disease from initial infection to abortion in pregnant females. Characterisation of strains implicated in bovine abortion will provide insights into the behaviour of the bacteria, and aid in our knowledge of bacterial dissemination and disease progression.

This study aims to describe the growth, virulence and antibiotic sensitivity of 15 circulating isolates derived from bovine abortions in 2017.

Sensitivity to antibiotics commonly used in the beef and dairy industries (tetracycline, streptomycin, chloramphenicol, amoxicillin-clavulanic acid, trimethoprim-sulfamethoxazole and nalidixic acid) was measured by disc diffusion assays. Virulence was investigated by infecting bovine caruncular epithelial cells (BCEC) for 2 and 24 hours. All assays were performed alongside the well-characterised strain 2229 as a comparison.

Consistent with current UK surveillance findings, all 15 clinical strains were sensitive to all antibiotics tested in this study. All *S.* Dublin isolates were able to infect BCECs and replicate over the course of 24 hours, and initial studies suggest differences between strains.

Ongoing survival assays in serum will aid in our understanding of systemic dissemination. Taken together, these studies will provide insights into the progression of the infection leading to colonisation of the placentome and subsequent abortion.

M4_2019_P41

Identification and characterisation of a phase-variable restriction modification system in CC9 *Enterococcus faecalis*

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Enterococcus faecalis is of increasing concern due to a rise in antibiotic-resistant, nosocomial infections caused by the bacterium. Phase variable Type I Restriction Modification System (RMS) allows the cell to rapidly alter the methylome and target sites of the restriction system, linked to phenotypic variation in many bacteria species. Our data shows the presence of one of two phase variable type I RMS in almost all isolates of many clonal complexes (CC) responsible for clinical infections, the *hsdR* and *hsdM* genes of the two systems are conserved where present, while the *hsdS* alleles are highly variable between (CC). Specifically, in the CC9 strain H25, we analysed the recombination frequency of the hsdS locus and isolated variants expressing the four hsdS alleles of the phase variable type I (RMS). We then utilised PACBIO sequencing to identify the target sequences of the four possible *hsdS* alleles of the phase-variable type I RMS. RNA-seq revealed several differential expressed operons between the variants and the presence of a differentially expressed long ncRNA that appears to have been acquired by two hyper virulent strains (H25 and Kb1)

M4_2019_P42

Estimating transmissibility of a hyper-virulent meningococcal clone

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Rapid transmission is a critical contributory factor in epidemics and outbreaks of invasive meningococcal disease. Rapid transmission requires a naïve population of sufficient size and intermingling. To examine precise population determinants of transmission, we examined the spread of a hypervirulent serogroup W clones in university student population where a 10-fold increase in carriage rate had been detected over a three-month time period. Spread of this clone occurred concomitantly with establishment of immunity due to the immunization of unvaccinated students as they arrived on campus. Examination of whole genome sequence data of 52 serogroup W isolates indicated the presence a number of phylogenetically-distinct clusters of varying size and diversity. These clusters were examined for the presence of genetic variation due to mutation and recombination, using a variety of dynamic high-throughput pipelines, enabling estimates of mutation and recombination rates during localised evolution of a meningococcal clone. Overall carriage rates and cluster sizes and distributions were utilized to estimate determinants of transmission. These compartmental models enabled the estimation of the number of contacts and the person-to-person transmission probability required for transmission of this hyper-virulent clone within the vaccinated population. These findings provide realistic estimates of spread of a hyper-virulent clone within a university population and will enable exploration of the level of pre-existing immunity required to prevent spread.

M4_2019_P43

Escherichia coli tightly regulates its internal free zinc concentration to a femtomolar concentration

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Background: *Escherichia coli* tightly regulates its internal free zinc concentration to a femtomolar concentration, this is achieved through tightly controlled gene regulation by two transcription factors; Zur and ZntR. ZntR is a zinc induced active activator, which regulates expression of ZntA, a P-type ATPase.

Aims: To develop a novel inducible promoter system, based on the zinc homeostasis *zntA* of *E. coli*. With the use of affordable zinc compounds as inducers. Further to this, to be able to finely tune expression levels of the system in response to inducer concentration.

Methods: Zur and ZntR regulated promoters were cloned into a dual reporter plasmid (pJI300), which encodes *mRFP1* and *luxCDABE* downstream of an MCS. Luciferase and florescent assays were conducted in *E. coli* MG1665 grown in zinc depleted Neidhardt's MOPS minimal media, and LB, with various concentrations of $ZnSO_4$.

Results: Luciferase assays showed significant reduction in promoter activity in the presence of 50 μM $ZnSO_4$ for four of the Zur regulated genes, as well as an increase in promoter activity in P_{zntA}. Both luciferase and florescent assays show P_{zntA} promoter activity increased directly with the increase of $ZnSO_4$ concentrations.

Conclusion: Results show promise for the use of inexpensive zinc compounds to be used as a cost-effective inducer. P_{zntA} shows increasing promoter activity with increasing concentrations of $ZnSO_4$, allowing for controlled expression level.

M4_2019_P44

Manipulating the macrophage: a role for the staphylococcal type VII secretion system

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The staphylococcal type VII secretion system (T7SS) is responsible for the export of several virulence factors important in staphylococcal survival within host cells. Some T7SS effectors are associated with long-term persistence of *S. aureus* abscesses in murine models and modulation of epithelial cell death, although the molecular mechanisms underlying T7SS mediated modulation of cellular processes remain unclear. The aim of this study is to investigate the role of staphylococcal T7SS during macrophage infection. Isogenic mutants lacking either single or multiple secretion substrates of the T7SS (EsxC, EsxB, EsaE and EsxD) or EssC, an ATPase driving T7SS protein export were studied in comparative *in vitro* analyses of bacterial entry, survival, macrophage cell death and cytokine/chemokine secretion profiles.

Over the course of a 24-hour macrophage infection *S. aureus ΔesxC*, *ΔessC* and *ΔesxC-D* mutants induce significantly less THP-1 cell and nuclear shrinkage when compared to the WT, as assessed by quantitative confocal microscopy analysis. Time-lapse microscopy revealed a marked contrast in how T7SS mutants and WT *S. aureus* escape from macrophages, with the WT inducing more pyroptotic or necroptotic-like swelling and lysis. Immunoblots for specific cellular markers of necroptosis and pyroptosis of infected macrophage lysates revealed levels of activated mixed lineage kinase domain-like protein (MLKL) and cleaved gasdermin-D (GSDMD) were differentially modulated when macrophages were infected with WT as compared to the T7SS mutants. We are currently investigating the activation of other markers in programmed cell. Our results suggest a role for the staphylococcal *T7SS* in reducing the execution of programmed cell death in WT infected macrophages enabling the bacterium to replicate and escape from cells during infection.

M4_2019_P45

Isoprene monooxygenase: A missing link in the global isoprene cycle

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Isoprene is a volatile organic compound (VOC) produced by many plants. Isoprene emissions account for one third of total VOCs from biogenic and anthropogenic sources, equal to that of methane. Atmospheric isoprene affects retention of greenhouse gases, air quality and climate. Recently, research has focussed on the role of bacteria in moderating isoprene emissions. Several diverse taxa of bacteria have been isolated capable of using isoprene as a sole carbon and energy source, including the model organism used here, *Rhodococcus* sp. AD45, a Gram positive bacterium isolated from freshwater sediment.

The ability to utilise isoprene is dependent upon a multistep pathway. The initial step is oxidation of isoprene to epoxy isoprene, catalysed by a four-component soluble di-iron monooxygenase (SDIMO), isoprene monooxygenase (IsoMO). IsoMO is a six protein complex comprising an oxygenase containing the di-iron active site (IsoABE), a Rieske-type ferredoxin (IsoC), NADH reductase (IsoF) and a coupling/effector protein (IsoD), homologous to the soluble methane monooxygenase and alkene/aromatic monooxygenases. We have demonstrated IsoMO activity with a range of alkene and aromatic substrates and explored the enzyme kinetics of IsoMO in whole cells.

We show the purification of homologously expressed IsoMO proteins from a *Rhodococcus* sp. AD45 strain lacking the isoprene metabolic gene cluster, and characterisation of the Rieske-type ferredoxin component. We can reconstitute active IsoMO to enable biochemical and biophysical characterisation of the complete enzyme complex as well as of the individual components. The knowledge gained allows further insights into the enzymatic basis for isoprene degradation in the environment.

M4_2019_P46

Comparing *att* Site Conservation with *Helicobacter pylori* Bacteriophage Excision Efficiency

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The human pathogen *Helicobacter pylori* colonises approximately half of the world's population and infection can lead to a range of gastric diseases. The World Health Organisation (WHO) placed *H. pylori* as a high-priority pathogen due to its prevalence, treatment difficulties and rising rates of antimicrobial resistance. The temperate bacteriophages in *H. pylori* are poorly characterised, most likely due few bacteriophages isolated due to a large number and diversity of restriction modification (RM) genes. This work aims to study the co-evolution between bacteriophages and their hosts the bacteriophages; focusing on bacteriophage *att* site conservation and the effect on bacteriophage excision, as well as identifying potential bacteriophage host strains based upon presence and absence of RM genes.

Genome analysis using Phaster of 466 *H. pylori* genomes showed 37 intact bacteriophages, ranging from 12-30 Kb in length. Approximately half of the bacteriophage were shown to have integrated in the Lipid A biosynthesis (*lpxD)* and the S-adenosylmethionine synthase (*metK*) genes. The conservation between the *attL*, *att*R, and *att*P were investigated in multiple intact bacteriophage sequences allowing for prediction of bacteriophage excision. Using qPCR, the frequency of non-induced bacteriophage excision was found to be one bacteriophage genome per 250 *H. pylori* genomes with 100% homology between *attL, attR,* and *attP.* Multiple SNPs between *att* sites show no detectable bacteriophage excision. Using 107 RM system genes, a presence/absence matrix of the RM genes the *H. pylori* genomes was built and compared to the presence/absence of bacteriophage, allowing to select bacteriophage acceptor strains for bacteriophage isolation.

M4_2019_P47 (No flash)

Molecular patterning of *Vibrio cholerae* chromosome segregation proteins

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Chromosome segregation is an essential and dynamic process that has to be coordinated in space and time with cell division. In bacteria, this is orchestrated by the Par (Partition) system that localizes and moves the replicated chromosomes apart. Despite recent progress in our understanding of the segregation of smaller genomes such as plasmids, the molecular mechanisms underpinning chromosome segregation remain elusive. The Par system consists of *parS* centromere-like sequences and two proteins, ParA and ParB. In *Vibrio cholerae*, each of its two chromosomes has a distinct Par system to independently control its segregation. The Par system of chromosome 2 is essential for *V. cholerae* virulence and viability, making it a prime target for new antibiotics. We found that ParA2 exhibits an asymmetric concentration gradient in the cell that shows remarkable pole-to-pole oscillations. However, the molecular basis of these protein oscillations in positioning and segregation of chromosomes remain unclear. We show that ParA2 binds DNA cooperatively in the presence of ATP. Using cryo-EM, we determined the structure of ParA2-DNA filaments. Our map showed new interactions at the dimer-dimer interface spanning the entire length of the ParA2 dimer, providing structural basis of higher-order oligomeric assembly and DNA-binding cooperativity. We found that ParA2 undergoes a slow conformational transition between non-binding to DNA-binding states, regulated by its cognate partner, ParB2. This time delay allows ParA2 to migrate and rebind the chromosome at the poles, leading to the continuous pole-to-pole cycling that incrementally translocates the chromosomal loci toward opposite cell halves for segregation.

M4_2019_P48

Horizontal Gene Transfer of Antibiotic Resistance Genes in the Gut Microbiome

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Antibiotic resistance is always due to the accumulation of chromosomal mutations or the acquisition of genes that confer resistance. These genes are of particular concern as they can be shared between bacteria through horizontal gene transfer (HGT), which can give rise to the rapid dissemination of antibiotic resistance determinants through bacterial populations. One ecosystem of particular interest is the human gut microbiome.

Current metagenomic profile methods fail to link resistance genes to their host bacteria. Thus, the aim of this study is to develop a method to identify bacteria and the genetic elements that carry important antibiotic resistance genes in the human gut.

To address this goal, faecal samples were collected from adult volunteers. DNA was isolated from these samples, after which high throughput qPCR was performed to detect resistance genes present within the sample. Once information has been gathered from these methods, epicPCR (Emulsion, Paired Isolation and Concatenation PCR) will be performed. Use of this method generated amplicons that are fusion products of antibiotic resistance genes to the 16S rRNA genes of their host bacteria. Ultimately, results from these experiments allowed us to identify the microbial reservoirs of antibiotic resistance genes.

M4_2019_P49

The Roles of Outer Membrane Vesicles of *Helicobacter pylori* in Pathogenesis

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Background: Outer membrane vesicles (OMV) are naturally produced from pathogenic and non-pathogenic bacteria during growth. They contain virulence factors and other bacterial membrane structures. OMV have been reported to have different roles in pathogenesis including packing and delivery of virulence factors into host cells, alteration of host immune response, and protection of bacteria in stressful environments.

Project aim: To characterise the roles of *H. pylori* OMV in pathogenesis by comparing between pathogenic and non-pathogenic strains in terms of OMV production and toxicity to mammalian cells.

Methods: *H. pylori* strains from patients with and without gastric disease were grown in BHI broth with 0.2% β-cyclodextrin and on blood agar plates for 24 hours. Produced OMV were purified by filtration and ultracentrifugation and characterised. OMV were quantified by BCA-protein assay and nanoparticle tracking analysis (ZetaView). The amount of OMV produced per bacterial cell was determined by Miles and Misra quantification of the bacteria. OMV toxicity on human gastric epithelial (AGS) cells was determined by CellTiter assay.

Results: There was no clear association between the quantity or toxicity of OMV produced, and the virulence of the bacterial strain. However, the method of culturing *H. pylori* did affect OMV production and characteristics. *H. pylori* produced higher quantities of OMVs in broth cultures than on plates. *H. pylori* strain 60190 and 444A OMV isolated from plate cultures were more toxic to AGS cells than those isolated from broth (****p).

Conclusion: Characteristics of *H. pylori* OMVs within a strain change depending on the environment in which the bacteria grow. This has potential consequences on the bacterial virulence.

M4_2019_P50

The involvement of heparan sulphate proteoglycans in tetraspanin mediated staphylococcal adhesion

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Colonisation of the epithelia by bacteria is a critical first step in pathogenesis. *Staphylococcus aureus* utilise heparan sulphate proteoglycans (HSPGs) to adhere to epithelial cells and induce proteoglycan shedding to increase virulence. Tetraspanin family members can associate with HSPGs and other adhesin receptors to form tetraspanin enriched microdomains (TEMs) on the host plasma membrane. Previously we have demonstrated that blockade of the tetraspanins significantly reduces staphylococcal adherence to epithelial cells. Here, we demonstrate involvement of the HSPGs in tetraspanin-mediated bacterial adherence. The human keratinocyte cell line, HaCaTs, were pre-treated with a CD9 tetraspanin-derived peptide, 800C, with or without HSPG blocking effectors and infected at an MOI of 50 for one hour. 800C and heparin sodium treatment significantly reduced staphylococcal adherence by ~60%, but no additive effect was observed in combination. Removal of heparan sulphates from the cell surface with heparinase demonstrated similar results while pretreatment of bacteria with heparin sodium abrogates the effect of the peptide. 800C was ineffective in an ADAM10-/- A549 cell line, similar to CD9-/- cells, indicating involvement of the metalloproteinase which initiates ectodomain shedding of HSPGs. Super resolution microscopy previously demonstrated that treatment of cells with 800C increases the size and area of TEMs. We hypothesise that increased spacing of TEMs reduces the efficient action of ADAMs on HSPGs and thereby reduces both virulence associated shedding and efficient binding to HSPGs as receptors. With significant reductions in bacterial adherence and virulence pathways, tetraspanin interference could provide a new pathway in the hunt for alternative anti-microbial treatments.

M4_2019_P51

Micro-scale topographies instruct bacterial attachment to surfaces

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Background: Topographical features fabricated with precise dimensions in the micro and nanoscale have shown some degree of success in reducing adhesion and bacterial biofilm formation. Most studies focused on a limited number of topographical designs, an approach that has restricted exploring model-based methods to draw correlations and predict bacterial responses based on surface properties.

Methods: To increase our understanding of the response of bacterial cells to surfaces a HTP process assessing bacterial adhesion to 2,176 distinct combinatorial generated micro-patterns (TopoChip) and real-time imaging of pili and flagella mutants were used to gain insights into the interplay between micro-topographical landscapes and bacteria.

Results: Specific substrate topographies have profound effects on bacterial attachment on different materials including relevant medical implant materials. The parameter fraction covered by features (size and density of micro-pillars) negatively influences bacterial adhesion in a reproducible mode. Monitoring of the spatio-temporal surface colonisation by *Pseudomonas aeruginosa* pili and flagella mutants provided insights into the resistance mechanism of lead topographies.

Conclusion: The high number of micro-topographies assessed and the remarkably strong correlation found between local landscape and bacterial attachment, allowed a detailed analysis on the relevance of surface parameters on adhesion and exploring an innovative approach for antifouling surface engineering, which predicts attachment based on surface design criteria rather than single traits such as surface energy or water contact angle. This illustrates the strength of unbiased screenings to reveal previously unperceived cell–surface interactions and provide insights towards the rational fabrication of new bioactive surfaces.

M4_2019_P52

Selection of efficient plant growth promoting rhizobacteria for the development of biofertilisers

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Agricultural practices depend on the application of chemical fertilisers to provide essential nutrients to plants. Nevertheless, the excessive use of chemical fertilisers may be source of negative environmental drawbacks such as soil, water and air pollution or decrease in soil fertility. To minimise these issues, a more sustainable and eco-friendly approach based on the application of biostimulants containing Plant Growth Promoting Rhizobacteria (PGPR) has been introduced in modern agriculture. PGPR represent a potential strategy to reduce the use of chemical fertilisers since they promote plant growth by supplying hormones and nutrients or increasing the uptake of elements from soils. Thus, the search for novel and suitable PGPR to be developed as commercial biofertilisers is becoming an important task for researchers and companies. In this work, bacteria were isolated from the rhizosphere of tomato plants (*Solanum lycopersicum* cv. Marmande) and were then selected for their ability to solubilise inorganic phosphate. Phosphate-solubilizing bacteria were subsequently identified through 16S rDNA PCR using universal primers (27F and 1492R) and screened for other plant growth promoting activities (i.e. potassium solubilization). PGPR belonging to *Pantoea* and *Pseudomonas* genera with more than one plant growth promoting activity were selected. Current investigation is now focused on synergism between these strains and other PGPR to develop a new generation of biofertilisers based on microbial consortia.

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M4_2019_P53

The role of CheA in *P. aeruginosa* surface sensing pathway

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Implanted medical devices are lifesaving tools in clinical settings, but they also promote bacterial infections. Over 1 million cases of catheter-associated urinary tract infections (CAUTIs) are reported every year worldwide. CAUTIs are caused due to the ability of the bacteria to attach to the catheter surface and form biofilms.

High-throughput screens of over 20,000 polymer and copolymer formulations prepared from commercially available acrylate monomers led to the discovery of novel class of biofilm resistant polymers (RPs). The RPs prevent irreversible attachment and biofilm formation. The mechanism(s) by which the bacteria fail to form biofilms on the polymer surface is not yet fully understood but it is likely to involve flagellar and type IV pili mediated motility.

The *P. aeruginosa* flagellar stators MotAB and MotCD are flagella motor components capable of responding to different mechanical loads. It has been proposed that the flagellum is a mechanosensor. Interestingly, experiments conducted in our laboratory indicate that *P. aeruginosa* MotAB and MotCD mutants only weakly attach to the polymer surfaces and may play a crucial role in bacterial cell decisions to stay or leave the polymer surface. The present study investigates the role of the histidine kinase CheA, which is encoded in the same operon as *motCD*, in the surface sensing pathway. CheA also regulates c-di-GMP cellular levels. This secondary messenger has been described to be upregulated during surface adaptation and during biofilm formation. Using RT-PCR, we show that *cheA* is part of a 9.01 kb operon that starts at gene *fleN* and stops before PA1462. In addition, a *cheA* mutant is unable to swim or to produce an EPS biofilm matrix in vitro or in vivo when compared with the wild type strain.

M4_2019_P54

Investigating the secretion of the serine protease autotransporter EspC from enteropathogenic *E. coli* using fluorescent tags

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The Autotransporter family of proteins includes virulence factors produced by numerous Gram-negative bacterial pathogens. Previously, we have shown evidence that the enteropathogenic *Escherichia coli* autotransporter EspC (EPEC-secreted protein C) localises into a spiral along the bacterial cell during secretion. This spiral localisation resembles the localisation of the cytoskeletal shape-forming protein MreB, as well as the Sec translocon (Sec). Disruption of MreB or Sec resulted in an altered EspC localisation. Here we show colocalisation of EspC-mCherry and MreB-FlAsH in *E. coli* MG1655. To confirm the FlAsH-EDT2 substrate enters *E. coli* and specifically interacts with a CCPGCC motif, this motif was incorporated into cytoplasmically localised mCherry. As a ΔSecA mutant exhibited a bipolar localisation of EspC-mCherry, EspC localisation was monitored in three more temperature-sensitive Sec mutants (ΔSecY, ΔSecD and ΔSecF). To discount the possibility of the temperature shift having an effect on localisation, MG1655 was cultured at the non-permissive temperature of the mutants (25°C or 42°C) as well. EspC localisation and secretion was altered in the different mutants to varying extents, supporting a role of Sec in the spiral localisation of EspC. These results suggest close interaction of EspC with MreB and Sec during secretion. By elucidating the secretion mechanism and protein interactions of EspC, we hope to gain insight into autotransporters of related enteric pathogens to underpin the development of new antimicrobials.

M4_2019_P55

A *rsaM* homolog regulates quorum sensing and virulence in *A. baumannii*

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

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

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

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

M4_2019_P56

Measuring and Mapping real-time Oxygen Consumption in *Pseudomonas aeruginosa*

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The presence or absence of oxygen is important for both the establishment and growth of microbial biofilms as well as the effectiveness of antimicrobial treatments applied to treat infections.

Using oxygen sensitive, polyacrylamide nanosensors, real-time consumption of oxygen was tracked during planktonic growth by measuring changes in fluorescence intensity using a microplate reader. Whilst there was no obvious difference between the growth of two different lab strains of *Pseudomonas aeruginosa* (PAO1-N and PA14) when a range of nutrients were supplied exogenously in M9 minimal media; oxygen consumption was noticeably reduced in PA14. This observation hints towards the efficiency of PA14 to metabolise and grow using less oxygen than PAO1-N. This would be consistent with providing an evolutionary advantage to PA14 in a microaerophilic cystic fibrosis lung such as it was isolated from.

The sensitivity of the nanosensors to detect oxygen consumption during biofilm formation was compared against commercially available oxygen sensing products, such as PreSens OxoPlates. Further work is required to determine the effectiveness of the nanosensors under both static and flow-cell systems in order to characterise and map oxygen microenvironments within biofilms.

M4_2019_P57

Evaluation of the effects of the nitrogen fixing bacterium *Gluconacetobacter diazotrophicus* on tomato plants in hydroponic applications

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Gluconacetobacter diazotrophicus (Gd) is a non-nodulating endophytic nitrogen-fixing and phytohormone-producing bacterium capable of colonizing a wide range of crops and providing beneficial effects to the plant.

In this study, a hydroponic setup was used as an application strategy on tomato seedlings. A WT strain and a nitrogen fixation impaired strain ($nifD^-$) of Gd were investigated for their plant growth promoting effect. Tomato plants were grown with known amounts of KNO_3 (0 mM, 2 mM, 10 mM KNO_3) dissolved in the nutrient solution. Money Maker seeds were surface sterilised and grown for 14 days in growth chamber. Plantlets were root dipped for 72hrs in bacterial cell suspensions of WT or $nifD^-$ strain and then incubated in the growth chamber for 14 days. At 0 mM and 2 mM KNO_3, untreated (U.T.) and $nifD^-$-inoculated plants showed no significant difference in chlorophyll content and shoot length, while WT-inoculated plants showed higher chlorophyll content and shoot length in comparison to U.T. and $nifD^-$-inoculated plants. At 10 mM KNO_3, $nifD^-$-inoculated plants displayed higher chlorophyll content and shoot length in comparison to U.T. plants but WT-inoculated plants showed higher chlorophyll content and shoot length in comparison to U.T. and $nifD^-$-inoculated plants.

This study indicates a positive effect of *G. diazotrophicus* on tomato plants, in terms of chlorophyll content and shoot development. This positive effect is related to the nitrogen-fixing ability, since a reduced beneficial effect was observed in $nifD^-$-inoculated plants in comparison to WT inoculated plants.

M4_2019_P58

PA2384 is a regulator of pyoverdine production and is modulated by the Rsm system of post-transcriptional control

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PA2384 is a predicted Fur-like hypothetical protein in *P. aeruginosa*, the expression of which is elevated in iron limiting conditions. Previous work had shown that this gene is also highly expressed in the presence of the 2-alkyl-4-quinolone (AQ) quorum sensing signal molecule, PQS. This finding was further investigated using a range of constructed chromosomal deletion mutants, expression plasmids and transcriptional and translational reporters. Since PQS is a signal that can bind iron, we tested PA2384 expression against a range of PQS analogues- expression of PA2384 was shown to be most sensitive to PQS, however provision of iron dampened the PA2384 response. A Fur-titration assay demonstrated that PA2384 expression is under the control of Fur and therefore repressed in the presence of iron. Phenotypic analysis of a PA2384 mutant showed no effect on *pqsA* expression or AQ production, and no effect on pyocyanin, elastase, rhamnolipid or biofilm production. PA2384 had no effect on *pchR*, *pchD* or *pchE* pyochelin gene expression, however PA2384 negatively regulated the expression of both *pvdE* and *pvdS* pyoverdine gene expression, with corresponding effects on pyoverdine production by the bacterium. The promoter region of PA2384 also contains several predicted A(N)GGA binding sites for the Rsm post-transciptional control system. Translational fusions demonstrated that PA2384 expression is under positive post-transcriptional control of RsmA, and EMSAs confirmed these predictions, demonstrating that RsmA and RsmN likely bind to the PA2384 transcript at these sites. RsmA was hown to be also indirectly modulated by PQS, via the action of the small, inhibitory RNA, *rsmZ*, which was induced in the presence of PQS. PQS therefore influences the expression of PA2384 via multiple transcriptional and post-transcriptional regulatory mechanisms to balance the production of pyoverdine under iron stress conditions.

M4_2019_P59

In the absence of disease, alveolar macrophages may act as a reservoir for *S. pneumoniae*

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Recent work in *in vivo* murine and *ex vivo* porcine models of infection has established the importance of an intracellular phase of pneumococcal replication within a subset of splenic macrophages (Ercoli, Nature Microbiology, 2018). This intracellular phase of, what is considered to be, an extracellular pathogen fundamentally changes our understanding of the pathogenesis of invasive disease and opens new options for therapeutic interventions in the treatment of these diseases. We have now explored the possibility that the survival of *S. pneumoniae* within alveolar macrophages may be a pre-cursor to pneumonia and subsequent invasive infections.

Broncho-alveolar lavage (BAL) samples were collected in Blantyre, Malawi, where the adult carriage rate is significantly higher than in the UK. Confocal microscopy of BAL samples from naturally colonised volunteers has shown detectable pneumococci within alveolar macrophages. Analysis using pneumococcal omni-sera has shown >40% of HIV negative volunteers to be positive for *S. pneumoniae* in their BAL fluid. In half of the positive samples, pneumococci were confirmed to be localised within the alveolar macrophages by confocal Z-stack analysis. Where capsule types have already been identified the results observed with pneumococcal omni-sera have been confirmed using capsule specific pneumococcal sera.

This finding suggests a fundamental shift in our understanding of the term carriage - nasopharyngeal versus alveolar within-macrophage carriage - and as a result the pathogenesis of community acquired pneumonia.

M4_2019_P60

Plant growth-promoting effect of the endophyte *Gluconacetobacter diazotrophicus* on rice and imaging of the plant-bacterial interaction.

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The endophyte *Gluconacetobacter diazotrophicus* (Gd) is a Nitrogen-fixing, plant growth-promoting bacterium (PGPB) isolated from sugarcane. In contrast to Rhizobia, it is able to fix Nitrogen under aerobic conditions, and interestingly it was shown to establish N_2-fixing symbiosis with a wide range of crops.

In this study, a patented Gd strain isolated by Azotic Technology Ltd has been tested in rice for its potentiality as a biofertilizer, and the colonization process has been monitored in order to shape a model of the plant-bacterial interaction dynamics. When grown in hydroponics, the Gd-inoculated rice cultivar BT7 shows an improved phenotype in terms of plant fitness and roots length. To confirm the presence of the endophyte within plant tissues and investigate the colonization process, a GFP/GUS-tagged strain was created. The fluorescent signal was primarily detected on the root surface and lateral roots emergence sites in the early stage of colonization, whereas bacterial aggregates were observed in correspondence to the root hairs during later colonization stages. To overcome the possible loss of the tagging plasmids due to the lack of selective pressure in the hydroponic system, two new tagging vectors have been assembled in order to deliver a GFP/dsRed::GUS::Km^R cassette into Gd genome and get a constitutively tagged strain. Furthermore, a vector was built for tagging the Nif operon, encoding for the Nitrogenase protein responsible for the N_2-fixing activity, in order to assess the N_2 fixation of Gd during its endophytic cycle.

These preliminary results show how Gd can positively affect the growth of rice by establishing a functional symbiosis, confirming the promising data about its in-field formulated application and the efficacy of this PGPB as a biofertilizer.

M4_2019_P61

Interactions of Arbuscular Mycorrhizal Fungi and winter wheat in contrasting cropping systems

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The benefits of arbuscular mycorrhizal fungi (AMF) as plant health improving symbionts with a broad range of plant species have been well documented in research and are more and more recognised in plant producing industry. However, the importance of AMF in agricultural systems has not been experimentally validated. The difficulty arises because a beneficial outcome from the sensitive symbiosis of plant and fungi depends not only on multiple environmental factors, but also the host genotype. This study investigates the interactions between AMF (native vs commercial inoculant), wheat varieties (long vs short straw), fertiliser types (Biogas Digestate, Cow Manure, mineral fertiliser, no fertiliser) and crop protection (conventional vs organic) by using a multifactorial split plot field experiment over two years. In both growing seasons, shoots and roots are harvested for biomass and root colonisation assessment at five key growth stages. First year results showed low impacts of fungal inoculation on grain yield, plant growth and health, but major effects of fertiliser and host genotype on AMF colonisation of roots. Highest colonisation rates were reached at flowering in non-fertilised wheat plants of the conventionally bred variety while all fertiliser applications decreased AMF abundance significantly. Ongoing experiments will analyse the AMF species composition in the harvested wheat roots by distinguishing between native and exogenous AMF strains.

M4_2019_P62

PIMMS based characterisation of genes essential for growth of fish derived *S. agalactiae*

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The aquaculture industry has rapidly expanded and intensified and now supplies around 50% of the world's food fish. In order to maintain high levels of productivity and food security, effective disease control and diversification of farming is essential. Streptococcosis is a highly infectious bacterial disease, caused by a number of bacterial species including *Streptococcus agalactiae*. It manifests as septicaemia and results in high mortality rates, in particular in trout and tilapia species. To identify genes that are essential for bacterial survival during fish infection, we generated a population of *S. agalactiae* strains using the pGh9:*ISS1* insertional mutagenesis system and characterised conditionally essential genes for bacterial survival using NGS sequencing and the bespoke PIMMS bioinformatic analysis program to map the genetic location of the mutations. A number of genes were absent from the mutant population which were identified as essential for bacterial cell wall synthesis, fatty acid metabolism, DNA replication, protein synthesis and bacterial replication. Approximately 15 % of these genes encoded for hypothetical proteins of yet unknown function. Genes FtsA, FtsY, FtsL, SepF were found to be essential for *S. agalactiae* growth and have previously been shown to be essential for bacterial division in other bacterial species. These genes and their related products may therefore be suitable targets for future antimicrobial development. This population of these *S. agalactiae* mutants provides the framework for future comparative analysis to identify genes related to survival in fish serum or against components of the fish innate immune system, which is currently under investigation.

M4_2019_P63

Grapevine endophytic bacteria displayed tolerance to the co-occurrence of drought and salinity stress

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Abiotic stress conditions, such as drought and salinity, are some of the foremost limiting factors for agricultural productivity. In this study, we investigated the effects of drought and salinity stress and their combination on four endophytic bacterial strains (32a, 727b, 11e and D7G) isolated from grapevine plants. Firstly, we determined their tolerance to drought and salinity stress and their combination. Secondly, we investigated how drought, salinity and their combination modulated auxin and biofilm production, swimming and swarming motility, endophytic colonization and plant growth promotion. Drought, salinity and their combination were induced using different concentrations of polyethylene glycol and sodium chloride, alone or in combination. Bacterial strain 32a was the most tolerant to salinity, drought and their combination and produced significantly more auxins and biofilm under individual and combined stress. Swimming and swarming motility of 11e was significantly higher than the other bacterial strains under individual and combined stress. Endophytic colonization and tomato plant growth promotion by bacterial strain 727b was stronger than other strains, under combined stress as compared to individual stresses. Salinity and drought tolerant plant beneficial endophytic bacteria characterized in this study will be further developed as biofertilizers to mitigate the negative effects of abiotic stresses on crop production.

M4_2019_P64 (No flash)

SCC Mobile Genetic Elements in *Staphylococcus epidermidis*

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The epidemiological success of methicillin-resistant *Staphylococcus aureus* USA300 has been associated with the presence of two mobile elements, the arginine catabolic mobile element (ACME) and the copper and mercury resistance (COMER). These two mobile elements endowing their host with resistance to copper, which has been related to fitness and survival within macrophages. As several studies found that ACME is more prevalent and exhibits greater diversity in *Staphylococcus epidermidis* while COMER had not been identified in *S. epidermidis* or other staphylococcal species, we aimed in this study to evaluate the presence of ACME and COMER and analyse their diversity in our *S. epidermidis* clinical isolates.

The genomic DNA of 58 *S. epidermidis* clinical isolates, collected between 2009 and 2018 in Aberdeen, was prepared followed by whole genome sequencing. Core-genome phylogenetic tree has been constructed using RAxML and genome based MLST typing showed that more than half the isolates belong to the clinically predominant sequence type 2 (ST2), these isolates have been found to split into two lineages in the phylogenetic tree. SCCmecfinder showed presence of SCC*mec* in the majority of isolates while comparative analysis identified ACME-positive isolates clustered in one lineage with most of them belonging to ST48. ACME mobile element showed high variation even within isolates of the same ST. COMER-like elements have been identified in one of the two major hospital adapted drug resistant ST2 lineage and showed high stability. To the best of our knowledge this is the first molecular characterization of the COMER element in *S. epidermidis* strain.

M4_2019_P65

Analysis of PA27NR and *nirQ* as mediators of the action of PqsE upon the *pqsA* promoter and the impact of the *las/rhlR* box reveals complex PQS circuitry.

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The Quinolone signal response protein PqsE plays a crucial role in *Pseudomonas aeruginosa* pathogenicity. Among others, It balances the production of the Pseudomonas quinolone signal "PQS" by repressing the *pqsA* promoter, it is required for the production of multiple PQS-dependent and PQS-independent virulence factors such as pyocyanin, elastase, rhamnolipids, and also participates in biofilm formation. Since PqsE is not likely to bind DNA, the biomolecular mechanism underlying its regulatory effect remains elusive. Based on promoter pull-down analysis performed at a different stage of growth of *Pseudomonas* and inducing *pqsE*, this study identifies and analyses two main candidates to mediate the action of *pqsE* upon the *pqsA* promoter: The hypothetical protein PA27NR and the denitrification regulatory protein *nirQ*. Furthermore, using deletion, site-directed mutagenesis and bioreporter assays we show that: 1) PqsE is still able to downregulate *pqsA* in the absence of the candidates. 2) In a *rhlR* mutant, *pqsE* appears to be an inductor of *pqsA* and finally, we show that 3) The *las/rhl* box, CTGTGAGATTTGGGAG, centred at -311 bp upstream of the *pqsA* transcriptional initiation site, is critical for the understanding of the regulation of this promoter and how its presence/absence can impact the PQS circuity. These results provide new insights into the regulatory relationships between *pqsE*, *rhlR* and the *pqs* operon, and demonstrate further the complex regulation of the *P. aeruginosa* PQS system.

M4_2019_P66

Disulfide bond formation underpins antimicrobial resistance in Enterobacteria

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The disulfide bond formation (DSB) system plays a central role in protein homeostasis, folding hundreds of proteins in the cell envelope of Gram-negative bacteria. Whilst the importance of this system for bacterial virulence is well established, its contribution to antimicrobial resistance is unknown. Here we discover that disulfide bond formation is necessary for the function of key antimicrobial resistance determinants in Enterobacteria. Resistance arising from the production of β-lactamases, mobile colistin resistance enzymes (MCRs) and RND efflux pumps is dependent on the DSB proteins and especially on the oxidase DsbA. Furthermore, chemical inhibition of disulfide bond formation leads to sensitization of multidrug-resistant clinical isolates to multiple classes of existing antibiotics. Thus, DSB system inhibitors are promising next-generation antibiotic adjuvants that can act as resistance breakers.

M4_2019_P67

Development of novel biofertilisers based on a better characterization of plant-endophytic bacteria/adjuvant interactions

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Chemical fertilisers are widely used in conventional agriculture and they cause possible environmental impacts. Plant growth promoting endophytic bacteria can internally colonize plant tissues without causing damage or eliciting defence responses. Biofertilisers are substances that contain living microorganisms, which are able to colonise the rhizosphere or the interior of the plant and to efficiently stimulate plant growth. Some publications recently highlighted the beneficial effects of the combined application of endophytic bacteria and humic acid (HA) substances on plant growth. However, there is a lack of knowledge on the molecular mechanisms of their combined interaction with the host. The aim of current project is to get insight into the molecular basis of the interaction between endophytic bacteria and tomato plants in the presence of HA, in order to improve the understanding on the mechanism responsible for plant growth promotion. In this study, three bacterial strains that endophytically colonise tomato plants were selected and they were able to promote tomato shoot length in the presence of HA. Molecular mechanisms activated in tomato plants in response to endophytic bacteria will be obtained by a transcriptomic study in order to further develop novel biofertilisers that meet farmers' expectations.

M4_2019_P68

M4_2019_P69

The Effect of *Staphylococcus aureus mprF* Gain-of-Function Mutation in Persistence via Increased Expression of Virulence Genes

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S. aureus bacteraemia is a major serious bacterial infection and is complicated due to persistence and failure to response to treatment by an appropriate antibiotic. It is unclear how *S. aureus* evades the host immune system and displays resistance to antibiotics treatment during persistent bacteraemia (PB).

The multiple peptide resistance factor (MprF) is a membrane protein that catalyses the synthesis of lysyl-phosphatidylglycerol reducing the negative charge of the cell membrane. There are identified gain-of-function (GoF) mutations within *mprF* that enhances its expression and correlate with an increase in the positive charge of the membrane and daptomycin resistance[1]. Our previous study (Richards *et al.*, 2015) showed that *mprF* GoF mutations in PB MRSA isolates were associated with daptomycin resistance and persistence virulence phenotypes such as increased bacterial fitness and immune evasion[2]. However, the role of MprF in PB is still not fully understood. The aim of this study is to investigate molecular mechanisms involved in the GoF *mprF* mutations that increased bacterial fitness and caused persistent phenotypes.

Proteomics and transcriptional analysis of PB isolates with *mprF* mutations showed an increased in the expression of virulence genes encoding proteins involved in immune evasion and adhesion. Our data indicates that MprF mediates the increase in the transcription level of virulence genes via impacting their regulators.

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M4_2019_P70

Towards understanding the function of ActA protein in *L.monocytgenes* growth and peptidoglycan remodelling

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Listeria monocytogenes is a food-borne bacterial pathogen and may lead to life threating disease. It has many virulence factors, allow the organism to cross the intestinal barrier and reach the blood. ActA is one of the major virulence protein which has a different role in the pathogenesis. Moreover, the peptidoglycan (PG) of *L. monocytogenes* could be remodelled to be suitable for different bacterial activities by peptidoglycan hydrolases.

Currently, it has been noticed that it has a role in PG biosynthesis and division inside the macrophages, but the exact mechanism is unknown. This study suggests that ActA has a lytic transglycolysis (LTgs) activity which controls PG biosynthesis and remodelling *in-vivo*. Moreover, it is essential for *L. monocytogenes* persistence during infection.

Different recombinant versions of ActA protein with 6xHis were generated. They were expressed, purified, and the identity was confirmed by mass spectrometry. Candidate catalytic residues are currently being identified by application of bioinformatics, and their function will be verified by site directed mutagenesis. Future experiments such as analysis of muropeptides released from PG by ActA, complementation studies and pull-down assays will shed light on the function of this protein in peptidoglycan remodelling. Finally, ActA interacts with other enzymes involved in peptidoglycan synthesis/remodelling and disruption of these interactions can be considered a novel tool for prevention of *L. monocytogenes* persistence.

M4_2019_P71

Molecular genetics of the virulence plasmids of pathogenic *Escherichia coli* O104:H4.

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In 2011 a large outbreak of enterohemorrhagic gastroenteritis and haemolytic uremic syndrome (HUS) throughout Europe resulted in almost 4,000 infections, 845 cases of HUS and 54 fatalities. This was due to a dangerous exchange of mobile genetic elements (MGE) resulting in a hybrid strain of *E. coli* O104:H4. This strain carried an unusual combination of EAEC- and STEC-associated virulence factors on a plasmid and phage respectively. In vitro the virulence plasmid has exhibited unusual stability under a wide range of stresses in the laboratory; contrasting with rapid plasmid loss in the human gut. Here, we begin to characterise maintenance systems present on the virulence plasmid, likely responsible for its unique stability, such as toxin-antitoxin (TA) systems involved in post-segregational killing. These may contribute to plasmid maintenance and therefore increased virulence. By isolating and expressing genes of interest, we will analyse their specific function. Preliminary results indicate surprisingly high levels of toxicity from a variant of a known TA system. Once characterised we aim to understand specific environmental conditions able to disrupt these systems ultimately resulting in plasmid loss. This atypical strain displayed heightened pathogenicity and provided unforeseen treatment challenges; therefore, we aim to further our understanding of MGE carriage in O104:H4 as a model to predict and combat future outbreaks of hybrid pathovars.

M4_2019_P72

Vaccine target, Factor H binding protein, is typically expressed as a non-processed precursor on the meningococcal surface

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Meningococcal surface lipoprotein, Factor H binding protein (FHbp), is the sole antigen of the Trumenba vaccine (Pfizer) and one of four antigens of the Bexsero vaccine (GSK) targeting *Neisseria meningitidis* serogroup B isolates. Maturation of FHbp was presumed to occur for all isolates. However, we found that 91% of UK serogroup B isolates contain single nucleotide polymorphism(s) in their FHbp signal peptide and in all isolates tested, processing including lipidation and signal peptide cleavage, was prevented. Processing was restored by correction of the SNPs. Whilst reduced binding to SecA, shown by bacterial 2 hybrid studies, resulted in retention of some of this preprolipoprotein in the cytoplasm, a significant portion was translocated with some accumulating in the inner membrane and periplasm and the remainder localised to the surface. For these isolates, we provide evidence for escape from processing by Lgt (shown from lack of lipid by palmitic acid labeling) and LspA (supported by studies using globomycin) with resumption of translocation and surface localisation involving Lnt and Slam respectively, revealing new insights into their roles. We demonstrate the critical importance of a particular polar amino acid residue in the signal peptide which dictates the processing fate of FHbp. Isolates expressing unprocessed FHbp showed no difference in ability to bind human factor H, as confirmed by Flow Cytometry. However the reduced surface abundance again shown by Flow Cytometry was reflected by reduced susceptibility to killing by anti-FHbp antibodies. Our findings bear relevance for Trumenba and provide important insights for other lipoprotein-based vaccines in development.

M4_2019_P73

Characterising the effect of circadian rhythm on splenic macrophage control of systemic infection

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Previous work has documented significant differences in the survival time of mice following intraperitoneal infection at different times along the circadian cycle in experimental models of sepsis caused by *Streptococcus pneumoniae*.

Recent work in our group has revealed that the spleen is the major organ determining the outcome of *S. pneumoniae* infection; whether infection is controlled or progresses to sepsis. After splenic uptake and clearance of S. *pneumoniae* from the blood, a small percentage of single pneumococci can resist killing and persist within a specific subset of splenic macrophages (CD169+). *In vivo,* we have observed that these single bacteria then replicate intracellularly and subsequently lyse the host cell, seed into the blood, and cause sepsis. In light of this observation, we would like to characterise the mechanisms by which circadian rhythm may affect this vital intracellular step and ultimately the outcome of infection.

Here, we demonstrate from preliminary data that mice infected with *S. pneumoniae* at 10am, during their resting state, show significantly increased survival time and decreased splenic bacterial loads at 6 hours post-infection when compared to those infected at midnight. Additional experiments carried out at 10am yielded a 99.9% reduction in blood bacterial counts in Balb/c after 4 hours, and a 99.9% reduction in counts in C57BL/6 after just 2 hours. Spleen and liver bacterial loads and bacterial localisation to subtypes of macrophages has been confirmed by confocal microscopy.

M4_2019_P74

Identification and characterisation of a nitrite reductase from *Pseudomonas aeruginosa* showing strong potential as an antimicrobial target.

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Pseudomonas aeruginosa utilizes a wide range of virulence factors to adapt to the host environment. With the antimicrobial pipeline drying up, understanding and targeting virulence factors for therapeutic development is an exciting alternative for the discovery of novel inhibitors of disease. An integrated genome-wide transposon mutagenesis screening approach was performed in *P. aeruginosa* PAO1-L using multiple *in vivo* disease models with the aim to identify new virulence factors required for infection. A mutant attenuated in the production of multiple virulence determinants using *in vitro* assays was identified. This mutant also showed severe attenuation using *in vivo* models with up to an 80% increased survival in murine chronic and acute lung infection models. The predicted protein coded by the mutated gene showed homology to nitrite and sulphite reductases. Using the purified protein in a methyl viologen reduction assay, we have shown that this gene encodes a nitrite reductase, operating in a siroheme and 4Fe-4S dependant manner. The preference for nitrite and the requirement of siroheme suggests that the product of this gene is an assimilatory nitrite reductase and hence we propose it to be named as NirA. Work is now on-going to understand how NirA contributes to virulence and to determine the crystal structure of this protein with a view to screen for novel inhibitors of this enzyme using a drug discovery platform available in our laboratories.

M4_2019_P75

Investigation of the pneumolysin operon in *Streptococcus pneumoniae*

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Streptococcus pneumoniae is the causative agent of multiple diseases, including otitis media, pneumonia, bacteremia, and meningitis. Pneumolysin, a member of the cholesterol-dependent cytolysins (CDCs), is encoded by the *ply* gene and is the only toxin of the bacterium. The structure and function of the pneumolysin toxin has been investigated in detail, but the regulation of the gene have not been elucidated. Furthermore, nothing is known about the three genes upstream the *ply* gene in the operon. The aim of this study is to modify the genes within the pneumolysin operon and nearby regions and to study their regulation of the pneumolysin using a haemolytic assay.

Mutant constructs for each gene of the four-gene *ply* operon, the *yebC* regulator and the transcribed intergenic region between *ply* and *yebC* were obtained by inserting a 1.3-kb cassette containing a wild type copy of the *rspL* gene, conferring streptomycin sensitivity, as well as a kanamycin resistance marker (*kan*). In addition, unmarked mutants were generated by replacing the cassette with a homologous sequence introducing an early stop codon. In order to test the haemolytic activity of the wildtype and mutant strains, haemolytic assays were performed using bile salt deoxycholic acid, to lyse all cells to release the pneumolysin. The results show an increased activity of pneumolysin for tow SPD1727 mutants and a decrease in mutants for the transcribed intergenic region downstream *ply*. These preliminary data indicate that some of the genes within the operon are involved in regulation of the pneumolysin activity and or expression.

M4_2019_P76

A fluorescence-based method to monitor AMR plasmid prevalence in biofilms using flow cytometry

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Background: Conjugative plasmids carrying antimicrobial resistance (AMR) genes can transfer horizontally between bacterial cells in both planktonic and biofilm lifestyles. These plasmids contribute significantly to the problem of AMR in Gram-negative bacteria. Studying the movement of AMR plasmids in real-world settings such as biofilms is therefore important to allow development of strategies to reduce AMR gene transmission. We are developing a system to assess plasmid transmission/persistence within biofilms at the single cell level. A previously established fluorescence system using *Klebsiella pneumoniae* Ecl8 donor cells carrying a *gfp*-labelled plasmid and Ecl8 recipients with a chromosomal *mCherry* tag will be used to detect conjugation using flow cytometry and microscopy.

Methods: To measure transmission/persistence in biofilms, a method was required to examine single biofilm cells using flow cytometry. To do this, a biofilm model was developed and a variety of disruption techniques were examined. Confocal microscopy was used to assess biofilm and cell disruption.

Results: Event rate, number of events, biofilm disruption and data analysis methods were optimised for obtaining/ measuring single cells from an Ecl8 biofilm using flow cytometry. Confocal microscopy confirmed that a biofilm was developed using our model. Effectiveness of biofilm disruption techniques was assessed by microscopy.

Conclusion: This method will provide an alternative means to track conjugation levels within bacterial biofilm populations providing factors such as disruption, dilution and number of events are considered.

M4_2019_P77

The role and regulation of the PlcR-PapR circuit in *B. cereus* G9241, the causative agent of anthrax-like illness

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Bacillus cereus G9241, a member of the *Bacillus cereus* sensu lato complex, was isolated from a Louisiana welder with a pulmonary anthrax-like illness and is closely related to *B. anthracis*.

Most members of the *B. cereus* group express PlcR, a pleiotropic regulator of secreted proteins allowing insect infection, which is activated by the peptide PapR at stationary phase of growth. However, in all *B. anthracis* isolates, the *plcR* gene is truncated. It has been proposed that the acquisition of AtxA, the mammalian responsive transcriptional regulator, was incompatible with the activity of PlcR, leading to selection for PlcR inactivation. Interestingly, G9241 encodes intact copies of both *atxA* and *plcR*. We hypothesise that a change in the PlcR-PapR regulatory network in G9241 has allowed the co-existence of *plcR* and *atxA* through temperature dependent suppression of the PlcR-PapR circuit at the time AtxA becomes active.

Here we investigated the temperature dependent activity of the PlcR-PapR circuit in G9241. Plasmid based transcription-translation reporter strains of PlcR and PapR have been used to study the transcriptional and translational activity at 25 °C and 37 °C. From microscopic analysis, we found that PlcR::GFP showed heterogeneous expression after 24 hours and 48 hours at both temperatures. Western blot analysis of PapR::GFP reporters confirmed that PapR was secreted at both temperatures, but higher amounts were seen at 25 °C.

M4_2019_P78

Development of a chronic *Pseudomonas* biofilm infection model in wax worms

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Background: The wax worm (*Galleria mellonella*) has gained acceptance as a pre-mammalian model for studying microbial infections. In this model, *Pseudomonas aeruginosa* infections are severely acute due to rapid mortality. The *P. aeruginosa* Gac/Rsm system is known to be key in the switch from chronic to acute infection. We hypothesised that knockouts of the Csr/Rsm system should display reduced mortality in the wax worm model resulting in a chronic biofilm-mediated infection.

Methodology: PAO1-N Rsm knockouts were used to infect *G. mellonella*. CFU counts were performed to determine if survival was due to clearance of infection and/or establish if proliferation occurred *in vivo*. Visualisation of infection was undertaken using *lux* expressing PAO1-N.

Results: Significantly increased survival was observed with Δ*rsmA* ($p < 0.0001$), Δ*rsmN* ($p < 0.0346$) & Δ*rsmAN* ($p < 0.0001$). CFU counts of Rsm knockout infected wax worms confirmed that clearance of the infection did not occur in the majority of wax worms, suggesting long term persistence of infection (> 72 h). Visualisation of PAO1 infection was carried out through bioluminescence detection.

Conclusion: We show that *P. aeruginosa* Rsm knockouts appear to produce a chronic-like infection in *G. mellonella*. We are currently validating this model through biofilm visualisation and the testing of known antimicrobial and anti-virulence compounds.

M4_2019_P79

Crystal structure of pyocin S3 reveals a novel inactivating protein clasp of the immunity protein on its DNase.

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Pseudomonas aeruginosa is the major cause of mortality in critically ill and immunocompromised patients in hospitals. It is very difficult to treat due to its multi-drug resistance and biofilm producing capabilities. *P. aeruginosa* produces bacteriocins called pyocins which are protein antibiotics used to kill competing strains. The S type pyocins are multi-domain, secreted, narrow- spectrum bacteriocins synthesized by *P. aeruginosa* with a cognate immunity protein that protects the producing organism against suicide.

In this study, the DNase domain of pyocin S3 was overexpressed in *Escherichia coli* B834 (DE3) and purified as a complex with the immunity protein or as the free DNase domain using a Ni-NTA column and gel filtration chromatography. Labelling with SeMet enabled the high resolution x-ray diffraction to be interpreted structurally. The activity of DNase was detected both *in vivo* and *in vitro* using an agar plate stab test and a plasmid nicking assay, respectively. Mutations were also introduced into the predicted active site of the pyocin S3 DNase.

The crystallographic analysis revealed that the pyocin S3 immunity protein adopts a novel octopus clasp of the cognate DNase domain. Activity assays demonstrated that Pyocin S3 is a Mg^{2+}- dependent DNase with a high killing efficiency. A key finding of the work is that the predicted active site of the pyocin S3 DNase does not adopt the typical HNH structure found in other bacteriocins.

M4_2019_P80 Withdrawn

M4_2019_P81

Evidence for phospholipid export from the bacterial inner membrane by the Mla ABC transport system

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The Mla pathway is believed to be involved in maintaining the asymmetrical Gram-negative outer membrane via retrograde phospholipid transport. The pathway is composed of 3 components: the outer membrane MlaA-OmpC/F complex, a soluble periplasmic protein, MlaC, and the inner membrane ATPase, MlaFEDB complex. Here we solve the crystal structure of MlaC in its phospholipid free closed apo conformation, revealing a pivoting β-sheet mechanism which functions to open and close the phospholipid-binding pocket. Using the apo form of MlaC we provide evidence that the inner membrane MlaFEDB machinery exports phospholipids to MlaC in the periplasm. Furthermore we confirm that the phospholipid export process occurs through the MlaD component of the MlaFEDB complex and that this process is independent of ATP. Our data provides evidence of an apparatus for lipid export away from the inner membrane and suggests that the Mla pathway may have a role in anterograde phospholipid transport.

M4_2019_P82

Promoter methylation regulating the differential gene expression in *Streptococcus pneumonia*

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A high-frequency phase variable epigenetic control mechanism in bacteria was discovered in the human pathogen *Streptococcus pneumoniae* and it was hypothesised that methylation by a type I restriction-modification (RM) enzyme would able to control gene regulation via epigenetic changes. In the pneumococcus the SpnIII RM system generates six different *hsdS* (host specificity determinant) alleles via site specific recombination resulting in the different methylation sites between single strains expressing stably a single HsdS variant. RNAseq was used to analyse multiple independent *S. pneumoniae* strains expressing only one of three single *spnIII* alleles (*spnIII*A, *spnIII*B or *spnIII*E) to determine any difference in gene expression profiles. The data have identified six genes which show differential expression and have a methylation site mapping to their predicted promoter. This study is aimed to investigate gene expression might be regulated by methylation of the nucleotide sequence of the promoters or regulator binding sites. To address this, six synthetic promoters with the wild type and altered methylation target site were cloned in front of a luciferase genes in *Streptococcus pneumoniae*. For two of them we have preliminary data (*ribD* gene and SPD_1532) which indicate that the methylated promoter shows higher promoter activity compared with non-methylated promoter. This result indicates that promoter methylation regulates the gene expression.

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