Understanding and predicting microbial evolutionary dynamics – 2025 meeting

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POSTER ABSTRACT BOOK

#MicroEvo25

MICROBIOLOGY SOCIETY

P001

Investigating competitive dynamics of ESBL E. coli against common E. coli using high-resolution lineage tracking

Inga-Katariina Aapalampi, Johannes Cairns, Teppo Hiltunen, Harri Savilahti, Manu Tamminen

University of Turku, Turku, Finland

Abstract

Antimicrobial resistance (AMR) is a global threat and multidrug-resistant ESBL *E. coli* in particular cause infections that are difficult to treat with antibiotics. The competitive dynamics of ESBL *E. coli* against commensal *E. coli* living in the human gut are not yet known. Understanding these dynamics explains how antibiotics and ecological interactions influence AMR evolution and competitive fitness.

Rapid evolution influences ecological dynamics, but it is unknown how molecular evolution is altered at increasing levels of ecological complexity. Experiments are mostly performed in one- or two-species systems, and molecular evolution in complex communities cannot be followed at high resolution with current research methods. With a new genetic engineering method called high-resolution lineage tracking (HRLT), it is possible to monitor strain frequency and the frequency of thousands of clades simultaneously to determine eco-evolutionary dynamics.

Here, we will develop and use HRLT technology to study eco-evolutionary dynamics of multidrug resistant ESBL *E. coli*. First, barcoded and strain coded bacterial clones will be created. Then, a three species community containing two common *E. coli* and one ESBL *E. coli* strain will be created. The community will be exposed to different antibiotics and antibiotic concentrations and 24-hour serial transfers will be performed for 20 days. The barcode locus will be amplicon-sequenced, and a large number of clones will be whole-genome sequenced. This will allow us to trace the competitive fitness of the strains and the evolution of mutations in the community. This data reveals the competitive advantage and evolutionary role of ESBL E. coli.

P002

Unlocking the Potential of Plasmid Dependent Bacteriophage PRD1 to Block Last Resource AMR Conjugative Plasmids

Michelle Yin, Daniel Cazares Lopez, Craig R. MacLean

University of Oxford, Oxford, United Kingdom

Abstract

The spread of AMR is mediated by plasmids, which uses conjugative machinery to facilitate the transfer of AMR genes across different bacteria isolates and species. As such, the conjugative pili is a potential target in the fight against AMR via the utility of phage therapy. The virulent phage PRD1 is able to target the conjugative pili of broad host range plasmids such as the canonical IncP in a wide range of bacteria, including critical pathogens from *Enterobacteriaceae*. PRD1 was previously shown to be able to impose selection pressure on conjugative plasmids, causing them to lose their conjugative ability. We performed a large screen of 65 PRD1-like phages against 11 AMR-relevant plasmids, which revealed that these phages have a larger plasmid host range than previously thought. With the genetic diversity present in our library, they can target beyond the known IncP1 plasmid, such as IncX4 plasmids. We developed conditions to capture better the range of plasmids that PRD1 can effectively target based on anti-conjugation assays and our results show that PRD1 can block the transfer of IncX4 plasmids as effectively as IncP1 plasmids, reducing transconjugants by 2-log, precluding the spread of colistin. The diversity in our panel will also allow us to conduct genetic screening and determine which specific genetic variation is responsible for the host range of the phage as well as verification by conducting a directed evolution experiment to expand to switch the host range of a smaller panel of PRD1-like phages.

P003

Thermal Adaptation and Mutation Dynamics in Sulfolobus acidocaldarius

Zahraa Albaqsami[1], Danna Gifford[1], Chris Knight[1], Rebecca Palmer[2], Andrew McBain[1]

[1]University of Manchester, manchester, United Kingdom. [2]University of Manchester, Manchester, United Kingdom

Abstract

Understanding how microorganisms adapt to temperature stress is essential for studying evolutionary mechanisms in extreme environments. In this study, I investigated the adaptive responses of three strains of the hyperthermophilic archaeon *S. acidocaldarius*: wild-type (DSM369), SK1 (*pyrE* & *Sual* knockout), and SK7 (*pyrE*, *Sual*, *nucS* knockout), evolved under four temperature regimes: optimal (75°C), suboptimal (65°C), supra-optimal (85°C), and a gradually decreasing condition. Fitness was assessed using growth rate and area under the curve at selected and non-selected temperature assays. Results showed strain- and temperature-specific adaptation patterns. Interestingly, several evolved lines from the strains displayed improved fitness at non-selected temperatures, with SK7 outperforming SK1 at 65°C assay temperature regardless of evolutionary background, indicating better low-temperature performance.

Whole genome sequencing revealed high-parallelism mutations in genes such as *tuf*, SACI_RS11220 and specific intergenic regions across selection conditions and strains. These shared mutations suggest potential convergent evolution and highlight a set of genes likely contributing to fitness in stressful environments.

P004

Prophage maintenance can have higher costs than phage resistance via cell surface modifications despite superinfection exclusion

Lavisha Parab, Amandine Dupuis, Carolin Wendling

Ludwig Maximilians Universität, Munich, Germany

Abstract

Infection by temperate phages can lead bacteria on different evolutionary routes, each with specific trade-offs. Becoming a lysogen – a bacterium with genomic prophages – prevents future infections through superinfection exclusion but comes with costs such as genomic maintenance of the prophage and risk of future cell lysis. On the other hand, mutations that modify the target receptor can grant phage resistance but are costly as they can disrupt its function. Here, we investigate the dynamics of naïve *E. coli* K-12 populations when exposed to either of four temperate phages. We find that populations diversify into lysogens and non-lysogens with non-lysogens outcompeting lysogens in most cases. Phenotypic screenings indicate that non-lysogens are resistant to phage infections; sequencing confirms the acquisition of surface receptor modifications. Our findings suggest that the costs associated with prophage maintenance may outweigh those of surface receptor modifications, diminishing the benefits of superinfection exclusion. To explore this hypothesis, we measure the competitive fitness of lysogens relative to non-lysogens in the presence (and absence) of different prophages. We also examine probable causes of the fitness differences, such as rates of (i) spontaneous phage induction, (ii) phage adsorption, and (iii) bacterial growth. Finally, a mathematical model integrates how these empirical parameters modulate the population dynamics. This study highlights how differential fitness costs arising from phage-bacteria interactions shape bacterial population structure, resulting in an evolutionary preference for surface receptor modifications over superinfection exclusion.

P005

CRISPR-Cas is beneficial in plasmid competition, but limited by competitor toxin-antitoxin activity when horizontally transferred.

David Sünderhauf[1], Jahn Ringger[1,2], Leighton Payne[3], Rafael Pinilla-Redondo[3], William Gaze[1], Sam Brown[4], Stineke van Houte[1]

[1]University of Exeter, Penryn, United Kingdom. [2]University of Basel, Basel, Switzerland. [3]University of Copenhagen, Copenhagen, Denmark. [4]Georgia Institute of Technology, Atlanta, USA

Abstract

Bacteria can encode dozens of different immune systems that protect them from infection by mobile genetic elements (MGEs). MGEs themselves may also carry immune systems, such as CRISPR-Cas, to target competitor MGEs, but it is unclear when this is favoured by natural selection. Here, we develop and test novel theory to analyse the outcome of competition between plasmids when one carries a CRISPR-Cas system that targets the other plasmid. Our model and experiments reveal that plasmid-borne CRISPR-Cas is beneficial to the plasmid carrying it when the plasmid remains in its host. However, CRISPR-Cas is selected against when the plasmid carrying it transfers horizontally, if the competitor plasmid encodes a toxin-antitoxin (TA) system that elicits post-segregational killing. Consistent with a TA barrier to plasmid-borne CRISPR-Cas, a bioinformatic analysis reveals that naturally occurring CRISPR-Cas-bearing plasmids avoid targeting other plasmids with TA systems.

Our work shows how the benefit of plasmid-borne CRISPR-Cas is severely reduced against TA-encoding competitor plasmids, but only when plasmid-borne CRISPR-Cas is horizontally transferred. These findings have key implications for the distribution of prokaryotic defenses and our understanding of their role in competition between MGEs, and the utility of CRISPR-Cas as a tool to remove plasmids from pathogenic bacteria.

P006

The impact of phage and phage resistance on microbial community dynamics

Ellinor Alseth[1], Rafael Custodio[2], Sarah Sundius[3], Rachel Kuske[3], Sam Brown[3], Edze Westra[2]

[1]University of Tromsø, Tromsø, Norway. [2]University of Exeter, Penryn, United Kingdom. [3]Georgia Institute of Technology, Atlanta, USA

Abstract

Where there are bacteria there will be bacteriophages, which are known to shape the microbial communities in which they are embedded. In turn, bacteria possess a range of distinct defence mechanisms that provide protection against bacteriophages, including the mutation of the phage receptor and CRISPR-Cas adaptive immunity. We previously showed how a microbial community may impact phage resistance evolution, yet little is known about how interactions between phages and these different defence mechanisms affect microbial communities. Here, we conducted a 10-day evolution experiment to examine how phage impact the structure and dynamics of an artificial four-species bacterial community that includes either *Pseudomonas aeruginosa* wild-type or an isogenic mutant unable to use CRISPR-Cas. Our results show that the microbial community structure is drastically altered by the addition of phage, with *Acinetobacter baumannii* becoming the new dominant species where *P. aeruginosa* would otherwise rule. Moreover, we find that *P. aeruginosa* with the ability to evolve CRISPR-based resistance generally does better when in the presence of *A. baumannii*, but that this benefit is largely lost over time. Finally, we hypothesise around why *P. aeruginosa* is unable to reinvade to become the dominant species in the community again, despite the phage being extinct. Combined, our data clearly illustrate how phage targeting a dominant species allows for the competitive release of the strongest competitor and contributes to community diversity maintenance and potentially preventing the reinvasion of the target species, while also highlighting how the interface between evolution and ecology is difficult to parse.

P007

Eco-evolutionary trait dynamics mediate the priming effect of disturbance pre-exposure on community resilience

Johannes Cairns[1], Niina Smolander[1], Sanna Pausio[1], Manu Tamminen[1], Ville-Petri Friman[2], Ville Mustonen[2], Teppo Hiltunen[1]

[1]University of Turku, Turku, Finland. [2]University of Helsinki, Helsinki, Finland

Abstract

Understanding how microbial communities respond to environmental disturbances is essential for applications ranging from sustainable agriculture to microbiota protection. However, the role of past disturbance exposure—"priming"—in shaping these responses remains underexplored. We used a synthetic 23-species bacterial community to experimentally dissect how ecological and evolutionary priming affects response to pulse disturbance by the beta-lactam antibiotic ampicillin.

We manipulated both the pre-exposure of community members and the presence of a pre-disturbance ampicillin pulse, and tracked community dynamics, resistance traits, genomic and transcriptomic changes, and antibiotic degradation over time. Community-level trait estimates showed that pre-pulses enriched resistant species, ecologically priming the community for increased resilience during the main disturbance. Evolutionary pre-exposure of member strains boosted baseline resistance in competitive species, reducing trait and compositional shifts during disturbance. Mass spectrometry revealed rapid ampicillin degradation, enhanced by pre-exposure, supporting an ecological mechanism of protection. Genomic analyses indicated that evolutionary priming reduced the number and diversity of de novo mutations during disturbance, while RNA-Seq analyses suggested altered gene expression profiles linked to pre-exposure history.

Our results demonstrate how ecological and evolutionary priming jointly shape community-level trait dynamics and disturbance responses. These findings highlight a generalizable mechanism whereby prior exposure conditions modulate community resilience through shifts in trait distributions and selective landscapes. Understanding such dynamics is critical for predicting and managing microbial community function under stress.

P008

Understanding and modelling protein evolutionary landscapes

Douglas Kell

University of Liverpool, Liverpool, United Kingdom. Danish Technical University, Lyngby, Denmark

Abstract

Synbio for biotechnology is synonymous with directed evolution as per the classical **D**esign-**B**uild-**T**est-**L**earn (**DBTL**) cycle, but now instead of 'blind' methods such as error-prone PCR we can create sequence diversity with much greater (statistical) control over the molecules or organisms made. While we can and do always measure the **objective function** (a yield or rate), cheap sequencing also tells us **where** we are in sequence space, and thus allows us to map, model and navigate the sequence-activity landscapes intelligently [1]. Having a model allows us to navigate the astronomically large search spaces using **active learning**, where we **choose** the next sequence to assess *in silico* [2]. The above applies whether we are engaged in enzyme, pathway, or organism engineering. We focus mainly on the first of these, where the nature of the search problem is especially clearly drawn [3, 4].

1 Currin, A., Swainston, N., Day, P. J. and Kell, D. B. (2015) Synthetic biology for the directed evolution of protein biocatalysts: navigating sequence space intelligently. *Chem Soc Rev*. **44**, 1172-1239. https://doi.org/10.1039/C4CS00351A

2 O'Hagan, S., Knowles, J. and Kell, D. B. (2012) Exploiting genomic knowledge in optimising molecular breeding programmes: algorithms from evolutionary computing. *PLoS ONE*. **7**, e48862. https://doi.org/10.1371/journal.pone.0048862

3 Kell, D. B. (2012) Scientific discovery as a combinatorial optimisation problem: how best to navigate the landscape of possible experiments? *Bioessays*. **34**, 236-244. https://doi.org/10.1002/bies.201100144

4 Kell, D. B. and Lurie-Luke, E. (2015) The virtue of innovation: innovation through the lenses of biological evolution. *J R Soc Interface*. **12**, 20141183. https://doi.org/10.1098/rsif.2014.1183

P010

Bacterial and plasmid interaction networks jointly shape community stability and evolution

Kaitlin A Schaal[1], Ying-Jie Wang[2], Johannes Nauta[3], Manlio De Domenico[3], Shai Pilosof[2], James PJ Hall[1]

[1]University of Liverpool, Liverpool, United Kingdom. [2]Ben-Gurion University of the Negev, Be'er-Sheva, Israel. [3]University of Padua, Padua, Italy

Abstract

There is a pressing need to understand the role of plasmids in shaping bacterial community dynamics. While studying individual plasmids and their fitness costs has taught us much, complexity theory argues that a full understanding of these processes cannot come from studying individual aspects in isolation. Our research therefore integrates interspecies interactions and plasmid interactions in a model soil community, using both modeling and experimental approaches. Our model predicts that plasmid host range can drive community composition; bacterial species that can host multiple plasmids (bridge species) are more likely to go extinct, even if the plasmids remain in the community hosted by other species. We tested this with a brief evolution experiment of a 3-species, 2-plasmid community, where each plasmid could be hosted by two species and one species could host both plasmids. We explored community dynamics across environments containing stressors relevant to resistance genes on the plasmids (kanamycin and mercury). We found that while the bridge species was unlikely to retain both plasmids simultaneously, it often persisted in the community. One species survived under MIC concentrations of antibiotic even in the absence of plasmids. Surprisingly, when this species carried a plasmid allowing it to survive the antibiotic, it was able to persist in the environment containing both stressors, even when the bridge species (which had access to both plasmids) went extinct. Overall, inter-species competition was often a more important driver in community composition than plasmid dynamics, although plasmid fitness cost did appear to contribute to species frequencies.

P011

Comparative evolution of antibiotic resistance across a diverse range of Pseudomonas species

Louise Flanagan, Magdalena Kurteu, Danna Gifford

University of Manchester, Manchester, United Kingdom

Abstract

Understanding how microbial communities respond to selective pressures can be challenging. By testing multiple single species against the same selective pressures, we can disentangle the factors driving variation in their responses.

In this study, we evolve a diverse range of Pseudomonas species—spanning pathogens and commensals from soil and human-associated environments—by gradually increasing their exposure to a panel of widely used antibiotics. This approach allows us to assess their capacity to develop resistance and uncover the genetic changes underlying these adaptations. We further examine the regulatory architecture surrounding these genetic changes, revealing how key regulatory features either promote or constrain resistance development.

By linking individual species' responses to antibiotics with their genetic and regulatory landscapes, this work provides critical insights into how these bacteria will perform in a natural microbial community. Our findings shed light on the genetic and regulatory signals that govern resistance emergence, improving our ability to predict when and how resistance will occur. This is important work to bridge the gap between controlled laboratory experiments and the dynamics of microbial resistance in real-world communities, advancing our understanding of microbial diversity and evolution.

P012

How do interactions between plasmids and transposons affect resistance gene spread?

Victoria Orr[1], Calvin Dytham[2], Ellie Harrison[3], James Halll[1]

[1]University of Liverpool, Liverpool, United Kingdom. [2]University of York, York, United Kingdom. [3]University of Sheffield, Sheffield, United Kingdom

Abstract

Horizontal gene transfer is a core driver of rapid bacterial evolution, and plasmids are key to this process, spreading adaptive traits e.g. resistance genes. It is increasingly clear that plasmid-borne resistance genes are usually 'nested' on transposons, enabling mobilisation and carriage by plasmids. This web of interactions (plasmid-transposon-chromosome) is predicted to have important implications for the spread of resistance genes. Using a laboratory microcosm system and modelling, we investigated how different plasmid 'vehicles' affected the spread of a chromosomal, transposon-borne resistance gene and how the transposability of a trait can affect single species populations. We found that resistance gene mobilisation varied across a panel of plasmids largely independent of conjugation rate or fitness cost, suggesting that other plasmid features, such as gene content or presence of other transposons, may influence chromosomal gene mobilisation. To test the contribution of intra-genome gene mobility to the persistence and spread of traits, we constructed plasmids carrying mobile (i.e. on a transposon) and non-mobile (i.e. integrated in the plasmid backbone) resistance genes, and tested the effects on MGE persistence under varying environmental selection regimes. To more broadly explore how plasmid features (conjugation rate, maintenance cost) combined with transposon features (transposition rate) affect resistance gene spread, we developed an agent-based model to disentangle the complexities of these interactions and predict how trait spread is affected in a variety of environmental scenarios. Understanding the potential outcomes and consequences of interactions between MGEs has important application in predicting the evolution of traits like antimicrobial resistance in microbial communities.

P013

A Multichannel Fluorescent Glycopolymer Array to Detect Pathoadaptations in Bacterial Infections

Kate Leslie[1], Sara Franco Ortega[2], Callum Johnson[1], James Moir[2], John Girkin[1], Ville-Petri Friman[3], Clare Mahon[1]

[1]Durham University, Durham, United Kingdom. [2]University of York, York, United Kingdom. [3]University of Helsinki, Helsinki, Finland

Abstract

Bacterial pathogens can evolve and diversify within hosts, leading to changing virulence factors such as antibiotic resistance and biofilm formation, complicating treatment. These pathoadaptations are difficult to detect even with state-of-the-art omics techniques, as multiple genetic changes can lead to the same phenotypic outcome. The opportunistic bacterial pathogen *Pseudomonas aeruginosa*, for example, causes persistent respiratory infections in patients with cystic fibrosis (CF). These infections cause progressive lung disease that severely limits life-expectancy, with the median age at death estimated to be 30.8 years.

We have developed arrays of diagnostic molecular probes using fluorescently-labelled glycopolymers that can discriminate *P. aeruginosa* strains based on the phenotypic variation in their surface properties. We have shown the interaction between glycopolymers and the surface lectins on bacteria can be linked to important pathoadaptations. This sensor array can distinguish genetically-engineered mutant strains differing in the expression of key virulence factors, as well as genetically variable *P. aeruginosa* isolates from CF patients sampled from different evolutionary lineages. We have further developed this array technology to require a reduced number of sensors, labelled with individually addressable fluorophores, which allows for single well analyte detection. Furthermore, we are examining the effects of concentration, and the presence of other bacteria species in mixed samples, to mimic real-life cases where infections are often polymicrobial. This array-based sensing approach could provide the underpinning technology for new diagnostic tools to map the evolutionary progress of persistent bacterial infections and inform treatment strategies.

P014

Experimental evolution of antibiotic resistance in standard and host-mimicking media

Charlotte Cornbill[1], Alasdair Hubbard[2], Freya Harrison[1]

[1]University of Warwick, Coventry, United Kingdom. [2]Nottingham Trent University, Nottingham, United Kingdom

Abstract

Understanding the evolution of AMR is integral to limiting AMR development and spread. Most laboratory AMR evolution experiments use standard laboratory media (e.g. cation-adjusted Mueller Hinton broth (caMHB)), but the environment can influence AMR evolution due to differing selection pressures and fitness costs. Here, we used experimental evolution to identify any differences in the adaptive landscapes of meropenem resistance in caMHB and two different host mimicking media.

Pseudomonas aeruginosa was selected for resistance to meropenem in caMHB, synthetic wound fluid (SWF) and synthetic cystic fibrosis sputum (SCFM) using an evolutionary ramp approach. Evolved clones were sequenced and their comparative fitness was assessed through antimicrobial susceptibility testing and growth curves.

SCFM displayed high meropenem resistance and high collateral resistance to ceftriaxone and levofloxacin, and increased amikacin susceptibility. SWF showed a similar resistance profile to caMHB, that had a smaller increase in meropenem resistance and collateral resistance to ceftriaxone and levofloxacin than SCFM. These differences were confirmed through different known resistance mutations across the three media. Despite SCFM clones being much more resistant, they showed minimal fitness costs when grown in the absence of meropenem. Whereas SWF and caMHB showed a large increase in lag time thought to be due to a mutation in the stringent response regulator, *spoT.*

The choice of media caused differences in resistance profiles and fitness costs. Future antimicrobial testing should take this into consideration as part of any translational work to move candidate drugs to the clinic and to better optimise our current antimicrobials in the clinic.

P015

The importance of social evolution for influenza infection outcomes

Rowan Green, Asher Leeks

University of British Columbia, Vancouver, Canada

Abstract

Social traits are those which impact the fitness of another such as cooperation, altruism and the division of labour. Although these phrases may conjure up images of worker bees or human societies, social traits abound in the microbial world. Cooperation is seen in viral populations where multiple virions frequently infect the same host cell, sharing products such as polymerase among all infecting genomes. Cooperation through the sharing of such public goods is vulnerable to cheating and viruses are no exception. Cheat genomes, in which the genes coding for these public goods are deleted, can benefit from reduced replication times while exploiting the public goods produced by the cooperating wildtype virus. In the laboratory, these cheaters can drive the viral population to extinction and have been successfully developed in vivo as therapeutic alternatives to traditional antivirals.

However, one outstanding question is whether naturally occurring cheats are similarly effective at combatting viral infections. Using a dataset of 200 human influenza A virus (IAV) samples we assess the relationships between cheat load and the clinical severity of the infection. The small genome size of IAV allows for deep sequencing of up to 250,000 reads per base. This reveals complex, heterogenous viral communities including multiple patients carrying over 100 unique cheat genotypes. By understanding the relationships between clinical outcomes and social evolution we can develop viral cheats as biomarkers for predicting clinical outcomes, allowing us to optimise the usage of limited antivirals. Furthermore, this will identify candidate cheat sequences for use as novel therapeutics.

P016

An unusual case of pathogen cohabitation in ash trees – adaptation to mutualism or bacterial cheating?

Robert Jackson[1,2], Katherine Hinton[1], Megan McDonald[1]

[1]University of Birmingham, Birmingham, United Kingdom. [2]Birmingham Institute of Forest Research, Birmingham, United Kingdom

Abstract

Ash trees in the UK are vulnerable to infection by the bacterium *Pseudomonas savastanoi pv. fraxini* (*Psf*). The disease is characterised by unusual erumpent cankers in the woody tissue and is widespread throughout the British Isles. Given the devastating impact of ash dieback on ash populations, we examined *Psf*-ash interactions, in case the fungal disease might drive changes in *Psf*-caused disease. Strains of *Psf* were isolated from six sites in England and Scotland. Genome sequence analysis of 130 strains identified 833 variant sites in the core genome, clustering into nine clades, whilst phenotypic analysis (growth, motility, surfactant production) of 31 phylogenetically diverse representative strains revealed variations between isolates. The population from Wytham Woods exhibited patterns consistent with a recent clonal expansion, with very little genetic variation. Despite this, divergent motile phenotypes were observed even within the same tissue, suggesting pathogen evolution and cohabitation. Comparative genomics identified a range of mutations, with changes in the global regulators *gacAS* commonly observed. Deletion of *gacA* or *gacS* in two of the ash isolates confirmed their role in regulating motility and surfactant production. Moreover, mutants exhibited reduced *in planta* fitness compared to the motile wildtype, which was restored upon co-inoculation. This work represents one of the first reports of pathogen diversification and cohabitation within a host plant. The widespread nature of the disease and low impact on tree mortality, combined with the shift in certain *Psf* populations to cohabitation, might indicate that *Psf* is undergoing a shift towards a more mutualistic lifestyle.

P017

Ecology and evolution of phage resistance in two-speciescommunities of *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Doran Goldman, Charlie Mo

University of Wisconsin-Madison, Madison, USA

Abstract

Understanding how bacteria evolve resistance to bacteriophage (phage) infection in natural communities is a crucial goal for the design of phage-based therapies. Most *in vitro* studies of phage resistance have so far focused on the experimental evolution of a single phage and host pair, but resistance evolution may be constrained, sped up, or otherwise modified by the presence of non-host species in more diverse natural communities. To investigate how the presence of a strongly competitive non-host species impacts the evolution of phage resistance, we studied the *in vitro* infection of methicillin-resistant *Staphylococcus aureus* (MRSA) with a lytic phage in the presence of another pathogen, *Pseudomonas aeruginosa*. *P. aeruginosa* commonly co-occurs with MRSA in chronic wound and cystic fibrosis polymicrobial infections and antagonizes MRSA using a variety of molecular mechanisms, though the two species typically coexist *in vitro*. During phage infection in the absence of *P. aeruginosa*, MRSA rapidly evolves phage resistance and maintains high abundances for several passages, regardless of whether phage populations subsequently persist or decline. In contrast, when *P. aeruginosa* is present in co-cultures alongside phage, most MRSA populations rapidly decline to extinction, even after becoming phage resistant. Notably, in some experimental replicates where MRSA populations start at lower abundances compared to *P. aeruginosa*, MRSA maintains high abundances without becoming phage resistant, potentially due to lowered phage replication and killing in these conditions. These findings underscore the diverse impacts that a single non-host species can have on the evolution of phage resistance, depending on specific ecological conditions.

P018

Insights into Bacterial Point Mutations via Single-Molecule Tracking of DNA Repair Proteins

Rebecca Lowry-Palmer, Raveen Tank, Emma Wright, Rok Krašovec

The University of Manchester, Manchester, United Kingdom

Abstract

Genomic mutations drive the evolution of antimicrobial resistance in bacteria; strains with elevated mutation rates are more likely to generate resistance-conferring variants. Although the fluctuation assay is a powerful technique for determining microbial mutation rate, it provides only population-level data and does not account for cell-to-cell variation. In this study, we use super-resolution microscopy to analyse the single-molecule spatiotemporal dynamics of *E. coli* DNA repair proteins, specifically MutS, MutM, MutY, and MutT. These proteins play key roles in bacterial mismatch repair and mutation avoidance. Using single-molecule localisation microscopy we visualise DNA repair processes in individual bacterial cells growing in different environments. This approach offers real-time insights into the bacterial mutation process and its potential dependence on local microenvironments, allowing mutational heterogeneity within bacterial populations to be explored.

P019

Pandemic lineage emergence leads to collateral species-wide evolution

Elizabeth Cummins[1], Robert Moran[2], Rebecca Hall[3], Seungwon Ko[1], Keith Jolley[1], Alan McNally[3], Samuel Sheppard[1]

[1]University of Oxford, Oxford, United Kingdom. [2]The University of Sydney, Sydney, Australia. [3]University of Birmingham, Birmingham, United Kingdom

Abstract

The emergence and dissemination of *Escherichia coli* ST131 in the early 2000s is well-documented. This sequence type accounts for ~20% of extra-intestinal pathogenic *Escherichia coli* (ExPEC) infections globally and displays high levels of resistance to multiple antibiotics. However, the effect of the emergence of ST131 on the wider *E. coli* population remains unknown. Here we show that alleles dominant within ST131 have increased in prevalence in human-associated *E. coli* over the last 20 years. We found that exchange of genetic material has been largely intra-phylogroup, with significant enrichment of ST131-associated alleles observed in phylogroup B2 between 2000 and 2014. Specific alleles of 24 loci defy this phylogroup recombination barrier to increase in prevalence across the species, many of which are associated with mobile genetic elements (MGEs) involved in antibiotic resistance. Furthermore, we found that accessory genes were twice as likely to possess the dominant ST131 allele compared to core genes. Our results demonstrate how the temporal dynamics of the ExPEC landscape can potentially influence the genomic content of closely related *E. coli* populations that are not necessarily ExPEC themselves. We identify allelic exchange driven by MGEs, most likely influenced by the transmission of multidrug resistance regions and their accumulation in ST131. This work exemplifies how examining the wider context of a pandemic lineage can provide further insight to the evolutionary processes and genetic hallmarks of successful pathogens.

P020

Impact of Microbial Interactions on the Fitness Costs of Antibiotic Resistance Mutations in *Pseudomonas aeruginosa*

Jack Knowles[1], Selina Lindon[2], Danna Gifford[3], Craig MacLean[2], Simon Cameron[4], Rachel Wheatley[1]

[1]School of Biological Sciences, Queen's University Belfast, Belfast, United Kingdom. [2]Department of Biology, University of Oxford, Oxford, United Kingdom. [3]Infection and Genomics, School of Biological Sciences, The University of Manchester, Manchester, United Kingdom. [4]School of Biological Sciences, Queen's University Belfast, Belfast, United Kingdom

Abstract

Global health continues to be threatened by antibiotic resistance, reducing our ability to treat infectious disease, leading to increased morbidity and mortality. Antibiotic resistance mutations are often thought to incur a fitness cost, reflected in reduced competitive ability without antibiotics. These costs are thought to represent a major obstacle to the spread of resistance at an epidemiological scale. We investigated whether microbial interactions influence the fitness costs of antibiotic resistance mutations in the opportunistic bacterial pathogen *Pseudomonas aeruginosa*. A large-scale screen of over 400 fitness assays was used to characterise the fitness of spontaneous resistance mutations in *P. aeruginosa*, using a library of 36 mutants and 13 human microbiome bacterial and fungal species. The fitness of antibiotic resistant mutants was assessed in co-culture compared to monoculture conditions, revealing that most resistance mutation and microbial interaction combinations did not significantly alter fitness. Mutants exhibited significant fitness differences in only 19% of fitness assays driven by microbial interactions; 9% showing increased fitness and 10% showing decreased fitness. Fitness differences were most often associated with mutations related to cell wall synthesis. Efflux regulation mutations demonstrated a mixed fitness response depending on the microbe, with near-equal increases and decreases in fitness. These context-dependent fitness costs will be further explored to better understand interactions underlying these variations. Understanding the factors that influence fitness costs associated with resistance is crucial for predicting the likelihood of newly emerged resistance mutations spreading and, ultimately, could lead to novel strategies for combating resistance by manipulating these costs.

P021

Antibiotic-Mediated Legacy Effects: Does Antibiotic Exposure Affect Bacterial Community Functioning?

Juhani Rantanen[1], Teppo Hiltunen[1,2], Ville-Petri Friman[2]

[1]University of Turku, Turku, Finland. [2]University of Helsinki, Helsinki, Finland

Abstract

Antibiotic resistance research has traditionally focused on studying clinically relevant bacterial species in monocultures in the lab. This line of research ignores the fact that in the natural world bacteria are embedded within complex microbial communities, neglecting the effects of community context on antibiotic resistance evolution. Moreover, in addition to causing direct effects on individual bacterial species, antibiotics can fundamentally alter microbial community composition, diversity and functioning. We call these antibiotics-associated changes in bacterial communities antibiotic-mediated legacy effects (AMLEs).

To study AMLEs in bacterial communities, we first formulated a null model to describe this phenomenon, which assumes that there is inherent variation in antibiotic resistance among community members, and that competition is the most prevalent ecological interaction between community members in the absence of antibiotics. Antibiotic disturbance will reduce the effect of competition by driving susceptible species into extinction. In the absence of migration, the recovering community will mainly consist of resistant strains or species, resulting in diminished diversity and potentially reduced community functioning in terms of reduced nutrient cycling capacity, lowered community stability and increased susceptibility to pathogen invasions (dysbiosis). These predictions are tested in synthetic microbial communities exposed to several different antibiotics and by experimentally measuring antibiotic effects on community diversity and functioning.

In conclusion, our research suggests that the effects of antibiotic exposure should be viewed from bacterial community context, acknowledging potential long-term legacy effects of antibiotics on bacterial community functioning and dysbiosis in host-associated microbiomes.

P022 Please note this abstract has moved from P087

Exploring Tripartite Endosymbiosis with Diverse Algal Symbionts in *Paramecium bursaria*

Daniel Malumphy-Montesdeoca, Irma Vitonyte, Erika Hansson, Duncan Cameron, Michael Brockhurst

University of Manchester, Manchester, United Kingdom

Abstract

The living of one organism within another, endosymbiosis, has evolved independently multiple times across the tree of life and often includes interactions between vastly unrelated organisms. This phenomenon is central to our understanding of the evolution of emergent complexity in modern life and is best exemplified by the formation of mitochondria, which gave rise to the domain Eukarya. In natural settings endosymbioses occur in the context of complex and diverse ecosystems. However, when studied it is often limited to overly simplistic models that revolve around one-on-one interactions between two organisms, obfuscating much of the complexity and diversity seen in natural settings. For our understanding of endosymbiosis to progress, more nuanced models need to be developed.

Paramecium bursaria has emerged as a model for the study of primary and secondary endosymbiosis in eukaryotes. Its symbiosis consists of the ciliate host housing multiple species of algae, providing them with organic nitrogen in exchange for photosynthetic products. *P. bursaria* can hold multiple different species of symbiont simultaneously, the experimental opportunities this presents are yet to be fully explored. We exploit this naturally occurring phenomenon to produce a tripartite model whereby *P. bursaria* plays host simultaneously to *Micractinium conductrix* and the *Choricystis parasitica*. We have tested host uptake and retention of co-occurring symbionts, and future study will look at host-mediated control of symbiont with co-occurring partners. By increasing the number of selfish entities that simultaneously co-habit the host, we hope complexity seen in nature can be mirrored in a tractable system.

P023

Role of prophages in the evolution and spread of antiviral resistance in *Pseudomonas aeruginosa*

Josie Elliott[1], Anna Olina[2], Edze Westra[2], Anne Chevallereau[1]

[1]MMSB-CNRS, Lyon, France. [2]University of Exeter, Penryn, United Kingdom

Abstract

Bacteria are predicted to be outnumbered by their viruses – phages – 10:1. However, co-infection dynamics of multiple phages in one host is understudied. To protect themselves, bacteria can either mutate genes essential for phage life-cycles, or express genes designed to destroy/halt phages. These defence genes are often encoded on mobile genetic elements (MGEs) suggesting inter-MGE competition as a driver of the evolution of defence systems. Prophages (dormant chromosomally integrated MGEs) constitute a rich source of defence systems, thus a potential vector for the dissemination of defence genes in populations. Using a collection of prophages and virulent phage (which cannot integrate into the host genome) in *Pseudomonas aeruginosa* we have identified prophages that provide resistance against virulent phage lysis to their host. Some prophages provide broad resistance against many virulent phages, while others provide resistance to a few virulent phages in an environmentally dependent manner. Finally, some virulent phages are sensitive to a broad range of prophage strains. Interestingly, these virulent phages also display temperature sensitivity, suggesting more global aspects of virulent phage physiology that leave them vulnerable to prophage-mediated resistance. We are using representative prophage-virulent phage pairs to investigate how prophage-encoded defence genes impact the selection and spread of co-infecting phages in a bacterial population? Furthermore, how the virulent phage can co-evolve and evade these prophage-mediated resistance types? Prophage and co-infection are abundant in natural settings, thus understanding the evolutionary interplay between MGEs is important for therapeutic and agricultural uses of phage.

P024

Drivers and constraints of prophage persistence in natural communities

Karina Krammer[1], Anne Kupczok[2], Carolin Wendling[1]

[1]Ludwig-Maximilians-Universität, Munich, Germany. [2]Wageningen University & Research, Wageningen, Netherlands

Abstract

Prophages are widespread in nature, but if their costs outweigh the benefits, they are rapidly lost from bacterial populations *in vitro* [1]. However, it is unclear how long functional prophages persist within a specific bacterial host in natural microbial communities, and, in the case of stable persistence, which evolutionary modes drive the persistence.

To answer these questions we are conducting a longitudinal cohort study over 18 months to follow the *in vivo* evolution of *Escherichia coli* lysogens repeatedly isolated from human fecal samples. Ancestral clones of persisting strains were isolated and sequenced, revealing a varying number of prophages per strain. As the study continues, we will be able to compare the ancestral sequences with later time points. We also conducted fitness assays *in vitro* to compare the isolated native lysogens with non-native lysogens, which we constructed by isolating and inserting the native phages from the cohort study into different *E. coli* strains. We found that certain bacteria-phage combinations have higher fitness than others and that fitness generally decreases in non-native hosts. This supports our hypothesis that compensatory adaptation as well as natural compatibility both drive prophage persistence in natural communities.

This study aims to advance our understanding of prophage-bacteria relationships in natural communities and has implications for both ecological and medical contexts.

References

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P025

Working Towards the Identification of Proteins with a Post-Mortem Function in *Escherichia coli*

Lucy Blagden, Martin Cann

Durham University, Durham, United Kingdom

Abstract

After bacterial death, we hypothesise that specialised catabolic processes release nutrients into the extracellular environment to support the growth of other live cells. Thus, the nutrients are public goods which benefit the whole population. This post-mortem nutrient recycling mechanism can benefit a subset of bacteria, which have adapted to 'cheat' by utilising these public goods but not paying the energy cost of producing the catabolic enzymes. This study provides insight into our studies to identify proteins with a post-mortem function to produce such nutrients. Several methods were utilised in this work to identify cheats capable of exploiting nutrient catabolism from wild-type cells. The primary method used followed the competition method described by Gibson et al. (2025) (doi.org/10.1038/s41467-025-56761-6), which mixed wild-type and knockout *Escherichia coli* cells in a ratio of 97:3. Samples were taken at the start and end of the experiment, with fold change in the ratio of the knockout compared to the positive control (*Δlon*). Here, we have identified a cheat candidate, where the protein product of the knocked-out gene has an intracellular role in cytoplasmic protein recycling, similar to the confirmed cheat (Δ*lon*) reported by Gibson et al. (2025). The protein product of the knocked-out gene has a hypothesised role in post-mortem recycling due to its similarities with Lon. The candidate identified will direct further work into post-mortem nutrient recycling roles of the protein and other post-mortem mechanisms.

P026

Mutually Exclusive Routes to Multidrug Resistance are Driven by Pangenomic Background.

Lucy Dillon[1], James McInerney[2], Christopher Creevey[1]

[1]Queen's University Belfast, Belfast, United Kingdom. [2]University of Liverpool, Liverpool, United Kingdom

Abstract

Background:

Prokaryotic pangenome variation is not random but shows highly structured associations in gene content, likely driven by natural selection. How these pangenomic selective patterns relate to an organism's traits and phenotypes is still unclear, but it follows that the essentiality of any gene to a particular phenotype may also be subject to the wider genetic environment. Supporting this, we previously showed that predicting antimicrobial resistance (AMR) phenotype requires whole-genome context rather than individual AMR genes alone.

Methods:

We aimed to further understand the routes to resistance within and between species by combining pangenomics and machine learning, using *Pseudomonas aeruginosa* and *Escherichia coli* due to their clinical importance. The machine learning models revealed genes statistically linked to particular AMR phenotypes. We then searched for pairs of these key genes in networks evaluating gene presence within the pangenome (if genes are associated/disassociated).

Results:

We found that AMR genes were present within the core genome in both species. These genes often conferred resistance to multiple drug classes, suggesting multidrug resistance genes are core. We identified genes linked to multidrug phenotypes disassociated in *E. coli*, suggesting mutually exclusive routes to MDR phenotypes.We then compared *P. aeruginosa*'s pangenome network to *E. coli*'s, in which we identified that the gene pairs had different associations in the other networks. This highlights the importance of understanding the genetic context of AMR mechanisms to explore how AMR arises in different strains/species, which will be a key component to tackling the increase of AMR.

P027

In vitro and within-patient evolution of *Klebsiella grimontii* to piperacillin-tazobactam resistance reveals shared and distinct trajectories

Ellie Allman[1], Aakash Khanijau[2,3], Rachel Mcgalliard[2,3], Richard N. Goodman[1], Christopher M. Parry[1,3,4], Enitan D. Carrol[2,3], Fabrice E. Graf[1], Adam P. Roberts[1]

[1]Liverpool School Of Tropical Medicine, Liverpool, United Kingdom. [2]University of Liverpool, Liverpool, United Kingdom. [3]Alder Hey Children's NHS Foundation Trust, Liverpool, United Kingdom. [4]University of Oxford, Oxford, United Kingdom

Abstract

Experimental evolution of bacteria towards antimicrobial resistance (AMR) may inform antibiotic therapy by leveraging collateral sensitivity and fitness trade-offs. However, the similarities between *in vitro* AMR evolution and resistance dynamics within the human host remain poorly understood, as host-associated selective pressures are more complex.

Three *Klebsiella grimontii* isolates cultured from one patient with a recurrent bloodstream infection at Alder Hey Children's Hospital, Liverpool, showed increased piperacillin-tazobactam (TZP) resistance over four months due to a single nucleotide polymorphism (SNP) in the promoter of chromosomal β-lactamase $bla_{\text{OXY-6-4}}$. We investigated the effect of bottleneck size and environment on the evolutionary trajectory of AMR emergence in the laboratory and compared this to within-patient evolution. The susceptible ancestor was evolved in sub-inhibitory concentrations of TZP in LB broth, followed by growth on TZP-supplemented LB agar. This was repeated with different bottleneck sizes (0.1% and 5%) and a second environment: LB with 5% sheep blood (0.1% bottleneck). Resistant colonies were selected, and TZP susceptibility, fitness, β-lactamase activity, and genomes compared to the ancestor and *in vivo*-evolved isolate.

The $bla_{\text{OXY-6-4}}$ SNP seen in the patient-evolved isolate emerged in all 0.1% bottleneck mutants across both environments. The 5% bottleneck lineages exhibited greater genetic diversity. Other mutations varied between *in vivo* and *in vitro* lineages, indicating that bottleneck size and environment influence the mutational landscape.

This study highlights similarities and differences in laboratory-based evolutionary trajectories compared to within-patient evolution and emphasises the need for robust, physiologically reflective model systems and cautious translation of *in vitro* findings to the clinic.

P028

Modelling a biologically representative environment: does respiratory-mimicking media influence the evolution of meropenem resistance in *Pseudomonas aeruginosa?*

Lauren Pittaccio, Rachel Wheatley, Simon Cameron

Queens University Belfast, Belfast, United Kingdom

Abstract

The emergence and spread of antimicrobial resistance poses a considerable risk to human health and the ability to successfully treat bacterial infections. Conventional evolution experiments in which single-species cultures of bacterial pathogens are exposed to antibiotics in rich laboratory media have allowed us to establish a fundamental knowledge of antibiotic resistance mechanisms, but it is clear these laboratory cultures fall short of representing the complexity of the biological environments where infections occur. The use of infection-niche mimicking media provides a simple solution to bridge this gap and more accurately represent the nutrient environment of the infection niche, without the use of animal models. Here we tested the impact of infection-niche mimicking media, modelled on the environment of acute respiratory infection, on the evolution of meropenem resistance in *Pseudomonas aeruginosa*. *P. aeruginosa* is an opportunistic bacterial pathogen that can cause severe lung infections in immunocompromised patients. An adaptive laboratory evolution experiment was carried out to investigate the evolution of meropenem resistance in *Pseudomonas aeruginosa* in this respiratory-mimicking media compared to rich laboratory tryptic soy broth. Differences in the evolvability of meropenem resistance, as well as in the meropenem concentrations tolerated by endpoint isolates, were observed between respiratory-mimicking media and tryptic soy broth. Genome sequencing, minimum inhibitory concentration assays and fitness assays of the evolved endpoint isolates have allowed us to build a picture of the differences in meropenem evolvability and evolutionary trajectories by which resistance is achieved when using respiratory-mimicking media compared to rich laboratory media.

P029

Micromanaging: The architecture and evolution of host control in a microbial symbiosis

Erika Hansson[1], Fiona Savory[2], David Milner[2], Daniel Malumphy Montesdeoca[1], Irma Vitonytė[1], Thomas Richards[2], Duncan Cameron[1], Michael Brockhurst[1]

[1]The University of Manchester, Manchester, United Kingdom. [2]The University of Oxford, Oxford, United Kingdom

Abstract

Microbial endosymbioses are fundamental to ecosystems ranging from coral reefs to agricultural soils, yet the evolutionary dynamics that underpin their stability remain poorly understood. This is particularly true in nascent or facultative symbioses where partners have diverging fitness optima. Resolving how control is achieved—how hosts maintain beneficial symbionts and suppress exploitation—is key to predicting how symbioses respond to environmental change.

We address this using the tractable ciliate—algal model system *Paramecium bursaria—Micractinium conductrix*. In this system, symbionts reside in host-derived vacuoles and exchange photosynthate for nitrogen. Hosts dynamically regulate symbiont load in response to light level, which determines the cost–benefit balance of the symbiosis. Prior work identified two candidate host control pathways working in tandem: a “carrot” (nitrogen provisioning via the arginine–polyamine pathway) and a “stick” (digestion, signalled through glycan-sensing).

We experimentally disrupted these pathways through targeted chemical supplementation and tracked host and symbiont growth. Disrupting control impairs the host’s ability to modulate symbiont load, but with light environment-dependent consequences for host proliferation. These results suggest that the costs of losing symbiont control vary with environmental context.

By the time of the conference, we will have completed fitness assays to quantify net costs and benefits, and initiated experimental evolution to test whether hosts can evolve compensatory control. These findings will advance our understanding of how control architectures confer evolutionary robustness in microbial partnerships—and the conditions under which they may adapt, or collapse, under changing pressures.

P030

Enhanced metabolic entanglement emerges during the evolution of an interkingdom microbial community

Jan-Luca Ariens[1,2], Giovanni Scarinci[3], Victor Sourjik[1,2]

[1]Max Planck Institute for Terrestrial Microbiology, Marburg, Germany. [2]Center for Synthetic Microbiology (SYNMIKRO), Marburg, Germany. [3]Massachusetts Institute of Technology, Boston, USA

Abstract

While different stages of mutualism can be observed in natural communities, the dynamics and mechanisms underlying the gradual erosion of independence of the initially autonomous organisms are not yet fully understood. In this study, by conducting the laboratory evolution on an engineered microbial community, we reproduce and molecularly track the stepwise progression towards enhanced partner entanglement. We observe that the evolution of the community both strengthens the existing metabolic interactions and leads to the emergence of de novo interdependence between partners for nitrogen metabolism, which is a common feature of natural symbiotic interactions. Selection for enhanced metabolic entanglement during the community evolution repeatedly occurred indirectly, via pleiotropies and trade-offs within cellular regulatory networks, and with no evidence of group selection. The indirect positive selection of metabolic dependencies between microbial community members, which results from the direct selection of other coupled traits in the same regulatory network, may therefore be a common but underappreciated driving force guiding the evolution of natural mutualistic communities.

P031

Persistence, coexistence, and the costs of resistance in a multitrophic microbial ecosystem

Shane Hogle[1], Milla Similä[1], Iina Hepolehto[2], Teppo Hiltunen[1]

[1]University of Turku, Turku, Finland. [2]University of Helsinki, Helsinki, Finland

Abstract

Since the discovery of the first antibiotics nearly a century ago, humans have used these molecules to control microbial communities and treat disease. However, the widespread use of antibiotics has led to the evolution of antimicrobial-resistant (AMR) bacteria, now creating one of medicine's most pressing challenges. Many forms of drug resistance impart a significant fitness cost, suggesting that when the antibiotic is removed, resistance should disappear. However, this idea is at odds with field and epidemiological studies showing the long-term persistence of AMR bacteria in the environment. Compensatory evolution is one mechanism that accounts for this apparent discrepancy, but little is understood about how ecological mechanisms (e.g., trophic and competitive interactions) influence AMR persistence in the environment. Here, we investigate how top-down trophic control impacts the fate of costly AMR mutations in competitive synthetic bacterial communities in the presence of a bacterial consumer, the model nematode *Caenorhabditis elegans.*We demonstrate that top-down trophic interactions between nematodes and AMR bacteria enable resistant species with high fitness costs to persist in the absence of antibiotics when they would otherwise be eliminated through competitive exclusion. Surprisingly, nematode feeding assays suggest that resistance evolution in keystone species alters their palatability and nutritional value, indicating that changes in predator preference may contribute to the persistence of AMR bacteria. Our work contributes to a deeper mechanistic understanding of AMR persistence in the environment and provides a striking example of how trophic complexity may stabilize AMR bacteria in natural communities.

P032

Selective Host Environments Shape the Biofilm Genetic Landscape in Pathogenic and Commensal Escherichia coli

Elizabeth G. Aarag Fredheim[1], Zin Anwar[1], Miriam K. Karlsen[1], Anna K. Pöntinen[2], Einar Holsbø[1]

[1]UiT The Arctic University of Norway, Tromsoe, Norway. [2]University of Oslo, Oslo, Norway

Abstract

Biofilm formation is an important virulence factor in *Escherichia coli*. Genes across several functional classes contribute to this trait, including those encoding adhesins, matrix, and accessory virulence factors like siderophores. However, the genetic basis of biofilm formation and its role in ecological adaptation remain poorly understood at the population level.

We analyzed 3,438 isolates from human-associated *E. coli* Sequence Types (STs) adapted to different environments: ST10 (gut commensal), ST11 and ST21 (ruminant gut), and ST73, ST95, and ST131 (human urinary tract). A custom ABRicate database, built from a literature review, was used to screen for biofilm-associated genes with ≥80% identity and coverage. Gene prevalence patterns were assessed using clustered heatmaps and principal component analysis (PCA).

Most isolates (>70%) carried genes for key matrix components (curli, cellulose, and PGA), though PGA was absent in ST21. High-risk pathogenic STs (ST73, ST95, ST131) harbored significantly more biofilm-associated genes (Kruskal-Wallis, $p < 0.05$) than other STs. Variation in presence/absence (P/A) (5–80%) of genes encoding adhesins, accessory virulence factors, and transport systems was observed in 10-23% of genes in human-adapted isolates versus 3% in ruminant-adapted isolates. PCA showed distinct ST clustering (27% explained variance), with overlap among high-risk STs, suggesting a shared gene pool.

Taken together, human-adapted E. coli may rely on subpopulations with distinct P/A patterns of biofilm-associated genes as a bet-hedging strategy in the human host environment. However, distinct profiles separate commensal and pathogenic isolates. Our findings highlight how selective host environments may shape the genetic architecture of biofilm formation.

P033

Uncovering the evolutionary dark matter of integrons

Filipa Trigo da Roza[1,2], André Carvalho[2], Amalia Prieto[2], Paula Blanco[2], Ester Vergara[2], Rocío Lopéz-Igual[3], Modesto Redrejo[4], Álvaro San Millán[1], Melanie Blokesch[5], José Antonio Escudero[2]

[1]PBE, Centro Nacional de Biotecnología, CSIC, Madrid, Spain. [2]MBA, Facultad de Veterinaria, UCM, Madrid, Spain. [3]Instituto de Bioquímica Vegetal y Fotosíntesis, CSIC and US, Seville, Spain. [4]Departamento de Bioquímica, UAM, Madrid, Spain. [5]UPBLO, GHI-SV, EPFL, Lausanne, Switzerlan

Abstract

Integrons are genetic platforms that drive microbial evolution by capturing, rearranging, and expressing integron cassettes (ICs). Although they are best known for spreading antimicrobial resistance genes, integrons also encode a wide variety of functions—such as phage resistance or metabolic traits—yet most remain uncharacterized. The exploration of this large genetic reservoir and further microbial adaptation prediction is currently limited due to methodological challenges in detecting integrons.

Here, we present a phenotype- and sequence-independent tool that allows scalable capture, monitoring, and functional screening of ICs from both mobile and chromosomal sources.

We redesigned a class 1 integron to serve as a capture platform, incorporating *ccdB* or *sacB* as counter-selectable markers with an embedded integration site, *attI*. These markers enable the selection of recombinants, ensuring viability only if disrupted by incoming ICs. The platform presents a low escape mutant rate, thus providing a broad range for detecting cassettes.

We adapted this platform to the naturally competent bacterium, *Vibrio cholerae*, to enable direct DNA IC capture. This facilitated the creation of six different *Vibrio* spp. integron cassette chromosomal libraries (>60 ICs per genome).

To demonstrate our tool's applications, we aimed to identify new phage defense systems encoded within integrons. We challenged these libraries against bacteriophage T4 and successfully retrieved both recently identified phage-defense systems and a previously unknown IC-encoded defense gene from *V. vulnificus* chromosomal integron.

These results emphasize the importance of integrons as drivers of microbial evolution and provide a scalable method for predicting adaptive potential through these genetic platforms.

P034

Biofilm selection constitutively activates c-di-GMP synthesis by the bifunctional enzyme MbaA

Øyvind Myrvoll Lorentzen[1], Sören Abel[2,1], Pål Jarle Johnsen[1], Christopher Frøhlich[1]

[1]UiT The Arctic University of Norway, Tromsø, Norway. [2]Norwegian Institute of Public Health, Oslo, Norway

Abstract

Background:

Biofilms, in which microbes are encased within a self-produced matrix, represent the primary mode of microbial life. Yet, our understanding of the molecular mechanisms driving the biofilm adaptation remains incomplete.

Methods:

In this study, we experimentally evolved *V. cholerae* in pellicle biofilms and whole genome sequenced the evolved populations to identify genetic mutations. Mutagenesis and biofilm measurements were conducted to characterize the biofilm forming capacity of evolved variants. Finally, enzyme kinetic analyses of evolved MbaA variants were conducted to map the enzymatic activity of wild-type MbaA and evolved variants.

Results:

We identified mutations in the polyamine-regulated, bifunctional enzyme MbaA that alters its enzymatic activity in metabolizing the conserved second messenger cyclic diguanylate (c-di-GMP). These mutations occurred in both the c-di-GMP-producing GGDEF domain and the c-di-GMP-hydrolyzing EAL domain of MbaA. Mutagenesis studies and biofilm measurements demonstrated that these mutations increased biofilm formation compared to the unevolved parental strain and rendered the enzyme insensitive to external polyamine signaling. Enzyme kinetic analyses revealed regulatory crosstalk between these domains, where mutations in either domain enhanced the production of c-di-GMP by MbaA while concurrently reducing its hydrolytic activity.

Conclusion:

Our findings illustrate how evolutionary pressure can rewire existing enzymes, driving a transition from a planktonic to a biofilm-associated lifestyle. Ultimately, this study provides mechanistic insight into how bacteria can leverage the evolutionary potential of bifunctional c-di-GMP-metabolizing enzymes to facilitate biofilm adaptation.

P035

Epistasis arises from shifting the rate-limiting step during the evolution of the β-lactamase OXA-48

Christopher Frohlich[1], Adrian Bunzel[2], Karol Buda[3], Adrian Mulholland[4], Marc W. van der Kamp[4], Pål J. Johnsen[1], Nobuhiko Tokuriki[3]

[1]UiT The Arctic University of Norway, Tromsoe, Norway. [2]Max Planck Insitute, Marburg, Germany. [3]University of British Columbia, Vancouver, Canada. [4]University of Bristol, Bristol, United Kingdom

Abstract

Epistasis, the non-additive effect of mutations, can provide combinatorial improvements to antibiotic resistance development that substantially exceed the contributions from individual mutations. Yet the molecular mechanisms of epistasis remain elusive, undermining our ability to predict pathogen evolution and engineer biocatalysts. We revealed how directed evolution of the β-lactamase OXA-48 yielded highly epistatic activity enhancements toward the 3rd-generation cephalosporin ceftazidime. Evolution selected four mutations that increase ceftazidime resistance 40-fold, despite their marginal individual effects (≤2-fold). Synergistic improvements coincided with the introduction of super-stoichiometric burst kinetics, indicating that epistasis is rooted in the enzyme's conformational dynamics. Our analysis revealed that epistatic improvements in ceftazidime resistance stemmed from distinct effects of each mutation on OXA-48's catalytic cycle. The initial mutation increased protein flexibility and accelerated substrate binding, which is rate-limiting in the wild-type enzyme. Subsequent mutations predominantly led to improvements in the chemical step by fine-tuning substrate interactions. Our work identified an overlooked cause of intramolecular epistasis, where changes in the rate-limiting step can result in substantial synergy that boosts enzyme activity, and thereby antibiotic resistance.

P036

Integrases as Key Players in Shaping Pangenome Hotspots in Enterobacteriaceae

Romane Junker[1,2,3], Eduardo Rocha[1,2,3], Marie Touchon[1,2,3]

[1]Institut Pasteur, Paris, France. [2]Université Paris Cité, Paris, France. [3]CNRS, Paris, France

Abstract

Background

Mobile genetic elements (MGEs) often encode integrases that catalyze site-specific recombination between chromosomal target sites and MGE-associated sequences, contributing to bacterial genome diversification. In *Enterobacteriaceae*, genetic variability is concentrated in genomic hotspots that tolerate gene insertions while maintaining core genome structure. Many hotspots contain integrases, raising questions about the role of integration in shaping the composition and evolution of these regions.

Methods

To investigate the diversity and specificity of integrases in hotspots, we analyzed the pangenomes of 5655 complete genome assemblies from *Citrobacter freundii*, *Enterobacter hormaechei*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella enterica*. We developed a reproducible Snakemake workflow combining PanACoTa for pangenome construction, HMMER for integrase detection, SpotFinder for hotspot identification and MAFFT and IQ-TREE and phylogenetic analysis.

Results

We showed that hotspots containing integrases contributed ~75% of the accessory genome diversity, highlighting their role in pangenome evolution. Moreover, 70–90% of integrase gene families were exclusive to hotspots, suggesting strong site-specificity. We found that over 30% of identified integration hotspots were conserved across species, indicating that their origin can predate genus-level divergence. Furthermore, those homologous hotspots often encoded phylogenetically close integrases, showing a long-term preference of some integrases for specific genomic loci across lineages.

Conclusion

Our study reveals that integrase-mediated integration events have high target specificity and contribute significantly to pangenome evolution in *Enterobacteriaceae*. These integration hotspots likely act as adaptive platforms, facilitating gene flow across lineages. Future research should investigate the molecular mechanisms underlying hotspot conservation and integrase target specificity.

P037

Formation frequency of bacterial persisters and mutation rates in their progeny.

Ziang Zhang, Andrew Mcbain, Rok Krašovec

University of Manchester, Manchester, United Kingdom

Abstract

Persister cells are a major cause of antibiotic treatment failure and infection recurrence. While their ability to enter dormancy enables antibiotic tolerance, the mutational dynamics governing persister cells and their progeny remains poorly understood.

This raises the question of how the dynamics of persister cells change before and after their dormancy period. We examined this by testing persister generation frequency using different inducing treatments, followed by assessing mutation rates of persister progeny using fluctuation assays.

Our findings indicate that persister formation frequency largely remains similar (0.1% to 1%) across treatments including nutrient shift(glucose to fumarate), osmotic stress, heat shock and disinfectants, except for surprisingly high levels induced by DM to LB media transition which can go up to 70%. More interestingly, mutation rates of persister progeny remained identical to regular cells, contrary to our original hypothesis.

Advancing the understanding of the dynamics of persister cell formation is critical for identifying targets of clinical treatments, questioning the role of media transition induced cell cycle extension in persister formation alongside antibiotics. Wild-type mutation rates in persister progeny suggest that persister progeny retain identical genetic sequences to wild-type cells, confirming that persister formation relies on phenotypic switching rather than genetic mutations, with important implications for developing targeted therapeutic approaches.

P038

No individual effect does not mean no risk: Non-antibiotic drugs select for antibiotic resistance in mixture, but not individually

April Hayes[1], Lihong Zhang[1], Edward Feil[2], Barbara Kasprzyk-Hordern[2], Jason Snape[3], William Gaze[1], Aimee Murray[1]

[1]University of Exeter, Penryn, United Kingdom. [2]University of Bath, Bath, United Kingdom. [3]University of York, York, United Kingdom

Abstract

Antibiotic resistance genes can be selected for by a range of non-traditional antibiotic agents, including non-antibiotic drugs (NADs). Most commonly, quantifying selection for resistance is evaluated using single compounds, but the effects of mixtures can diverge from these single compound effects. Here, we tested the effects of diclofenac, metformin, and 17-beta-estradiol, commonly used NADs, individually, and in combination with ciprofloxacin, in a mixed bacterial community. Individually, these NADs do not cause large scale effects, but subtly changed the taxonomic composition of these communities, and selected for metal resistance (e.g. *arsB* and *arsR*), but not antibiotic resistance genes. However, when the same type of community was exposed to these NADs in combination with ciprofloxacin, we found that there was selection for multiple antibiotic resistance genes (e.g. *tolC*), and that these mixtures showed either an additive (17-beta-estradiol) or synergistic (diclofenac and metformin) response. Furthermore, we demonstrated that the communities exposed to the diclofenac and metformin mixtures responded in a similar way, whereas exposure to the 17-beta-estradiol mixture resulted in a divergent response. We propose that these mixtures have different mechanisms of action within this microbial community. Overall, we demonstrate the importance of considering mixtures when evaluating antibiotic resistance, and the importance of considering non-antibiotic agents. These mixtures can be more selective than the antibiotic alone, but the effects can often be subtle and include specific species or genes. Future research should include mixtures to better understand antibiotic resistance dynamics.

P039

Diversity and Resistance Trade-offs in *Pseudomonas aeruginosa* Isolates from Non-Cystic Fibrosis Bronchiectasis Patients.

Rana Alruthaya, Danna Gifford, Chris Knight, Michael Brockhurst

the university of manchester, manchester, United Kingdom

Abstract

Pseudomonas aeruginosa causes chronic respiratory infections that are difficult to treat because of its high phenotypic diversity and increasing antimicrobial resistance. While cystic fibrosis (CF) airways are well studied, less is known about *P. aeruginosa* evolution and resistance in non-CF bronchiectasis (NCFB). This study investigates baseline resistance profiles in 20 NCFB patients, using 1,800 isolates (90 per patient) collected before trial enrollment. Susceptibility was assessed for nine anti-pseudomonal antibiotics across six classes using the agar dilution method. Growth kinetics in antibiotic-free media were evaluated to determine resistance-associated fitness costs. Resistance co-occurrence patterns, MIC distributions, and variation across and within patients were analysed using network analysis, binary heatmaps, and mixed-effects models. Whole-genome sequencing of 345 isolates was also conducted to examine AMR genes and mutations. Fluoroquinolone (45.4%) and tobramycin (28.3%) resistance were common, while resistance to β-lactams and carbapenems was low. Resistance profiles varied widely, up to 25 distinct phenotypes per patient. Co-resistance networks revealed strong within-class associations and a negative correlation between fluoroquinolones and polymyxins, suggesting possible trade-offs. Fluoroquinolone resistance was associated with reduced growth; however, multidrug resistance showed minimal fitness costs. Genetic diversity did not consistently predict phenotypic diversity. These findings highlight extensive resistance heterogeneity in NCFB patients and the limitations of single-colony testing. Comprehensive profiling of multiple isolates may better inform personalised treatment strategies for chronic *P. aeruginosa* infections.

P040

Too Early or Too Late? Trade-Offs in the Timing and Specificity of Bacterial Defence Systems Against Mobile Genetic Elements

Paritosh Bedekar, Stineke van Houte, Edze Westra, Mario Recker

University of Exeter, Penryn, United Kingdom

Abstract

Horizontal Gene Transfer (HGT) is central to bacterial adaptation and evolution, enabling acquisition of genes that influence critical traits such as antibiotic resistance and virulence. HGT is mediated by Mobile Genetic Elements (MGEs) like plasmids, transposons, and bacteriophages. While MGEs can be beneficial, some – such as bacteriophages – are harmful, prompting bacteria to evolve diverse anti-phage defence systems (DSs). Over two hundred DSs have been identified to date, and a single cell, on average, carries five. This remarkable diversity has sparked interest in trade-offs associated with DSs, such as balancing effective protection against associated costs.

Here we focus on a trade-off involving the timing of DS activation and the ability to discriminate between MGEs. DSs that sense early infection signals – such as foreign DNA or replication-related events – can neutralise MGEs before they harm the host. However, these signals are often shared across diverse MGEs, making it difficult to distinguish between beneficial and harmful elements. Conversely, DSs that detect later-stage signals – such as phage proteins or packaging-related events – allow more acute discrimination but may activate too late to prevent cellular damage.

Using an Individual Based Model (IBM), we explore how ecological and evolutionary factors influence the fitness of early- versus late-acting DSs. We examine conditions under which one strategy outcompetes the other and whether mixed strategies emerge. Our results offer insights into the evolutionary pressures shaping DS diversity, elucidating how bacteria navigate the complex landscape of HGT and its implications for bacterial adaptation and evolution.

P041

Chromosomal resistance mutations facilitate acquisition of multidrug-resistance plasmids in *Escherichia coli*

Khadija-Siddiqa N. Hanga, Michael A. Brockhurst, Michael J. Bottery

University of Manchester, Manchester, United Kingdom

Abstract

Bacteria can gain multiple resistance mechanisms in a single step by the acquisition of multidrug-resistance (MDR) plasmids, but it is unclear how antibiotic selection during the acquisition of MDR plasmids affects the evolution of additional resistance mechanisms. Through conjugating separate ESBL- and carbapenamase-producing MDR plasmids into plasmid-naive *Escherichia coli* hosts we examine the effects of acquisition of a single plasmid or co-acquisition of multiple plasmids upon fitness costs, resistance and subsequent genomic adaptation. We show that acquisition of pOXA-48, encoding OXA-48 carbapenamase, is associated with highly variable fitness costs and levels of resistance to ertapenem in transconjugants independent of the presence of pLL35. This phenomenon was not observed during the acquisition of ESBL CTX-M-15 encoding pLL35 alone. Transconjugants receiving pOXA-48 rapidly gained parallel mutations affecting the membrane porin OmpF, or its regulators OmpR or EnvZ. These chromosomal mutations were not compensatory for the fitness costs imposed by the plasmid, nor did they provide significant increases in resistance to carbapenems in the absence of the pOXA-48. Rather, they acted synergistically with the plasmid-encoded carbapenamase, which alone only provided marginal resistance, together providing high-level resistance to ertapenem. Such rapid evolutionary processes may play an important role in plasmid dynamics within environments with strong antibiotic selection for plasmid-encoded ARGs, particularly when these ARGs provide only marginal resistance.

P042

Does bacterial cooperation facilitate the evolution of antibiotic resistance?

Rosie Randall

University of Oxford, Oxford, United Kingdom

Abstract

Antimicrobial resistance presents a severe threat to human health, with a predicted 10 million attributable deaths per year by 2050. A major mechanism of antimicrobial resistance, in both Gram-negative and Gram-positive bacteria, is the production of β-lactamases. β-lactamases are enzymes produced by bacterial cells to hydrolyse β-lactam antibiotics. Laboratory experiments have shown that β-lactamases can act as cooperative public goods and provide protection to neighbouring cells. Furthermore, population genomic analyses have detected signatures of kin selection in β-lactamase genes, in both *Pseudomonas aeruginosa* and *Bacillus subtilis*. However, the sub-cellular localisation of β-lactamases could affect the extent to which they protect neighbouring cells. For example, does a β-lactamase secreted into the extracellular space provide the same protection as β-lactamase in the periplasm? And does the strength of kin selection vary for β-lactamases with different sub-cellular localisations? Here, I use genomic tools and molecular population genetics to test whether β-lactamases show signatures of kin selection in 8 species of bacteria.

P043

A Model System for Investigating Predator–Prey Interactions in Synthetic Microbial Communities

Sanna Pausio, Teppo Hiltunen

University of Turku, Turku, Finland

Abstract

Maintaining and engineering functional, stable microbiomes in rapidly changing environments requires a deep understanding of microbial interactions and their responses to environmental change. Simple model communities in carefully regulated conditions can reveal fundamental principles of community assembly and function.

We have developed a panel of four ciliate predators and 23 bacterial species for studying community dynamics in a predator–prey system. The ciliates — *Tetrahymena thermophila*, *T. pigmentosa*, *T. pyriformis*, and *T. lwoffi* — can be maintained under laboratory conditions and feed on focal bacterial species. They differ in size and ecological characteristics. The prey community is a synthetic bacterial community of diverse origins. Member species vary in nutrient use, growth rate, and biofilm formation.

The bacterial community was co-evolved with and without *T. thermophila* for 151 weeks prior to monoclonal isolation, enabling the study of naïve and co-evolved subcommunities. Using these evolved isolates, we will assess the effects of (1) trait diversity and (2) evolutionary history on community dynamics in a two-trophic-level system through serial transfer experiments. We have monitored growth, community structure, and defense against predation. Bacterial growth and abundance were measured via optical density readings, and predator abundance using microscopic imaging and automated cell counting. Defense was assessed by measuring biofilm formation. Community structure will be revealed by sequencing a selected barcode region from the 16S rRNA gene for bacteria and the CO1 gene for *Tetrahymena*.

This study will contribute to our understanding of eco-evolutionary interaction networks in microbial communities.

P044

Fitness Effects and Transcriptomic Consequences of Carbapenemase-Encoding Plasmids in *Klebsiella pneumoniae*

Álvaro Barrera, Jorge Sastre, Alicia Calvo, Laura Toribio, Coloma Costas, Álvaro San Millan

National Center for Biotechnology, Madrid, Spain

Abstract

The spread of carbapenem resistance in *Klebsiella pneumoniae* poses a significant clinical threat. Two of these clinically relevant plasmids, pVIM-1 and pOXA-48, have been found in multiple nosocomial outbreaks. These plasmids belong to the plasmid taxonomic unit L/M (IncL), and present a similar background, but encode different AMR genes. While the role of these plasmids in resistance acquisition is well established, their impact on bacterial host physiology remains poorly understood. In this study, we investigated the fitness and transcriptional effects associated with the acquisition of pVIM-1 and pOXA-48 across ten *K. pneumoniae* clinical strains belonging to two of the most relevant sequence types (ST) in hospital settings: ST11 and ST307. We constructed three versions of each strain: carrying pOXA-48, carrying pVIM-1, or carrying none of them. We measured plasmid-associated fitness effects using growth kinetics and competition assays. Our results revealed substantial strain-dependent variability in fitness effects, with some strains experiencing substantial growth disadvantages, while others being minimally affected or even enhanced by the plasmid. Both plasmids showed similar behaviours in the same strain, highlighting the importance of genomic background in plasmid maintenance.To uncover the potential molecular mechanisms underlying these differences, we performed transcriptomic analysis comparing plasmid-carrying and plasmid-free strains. Differential expression analysis revealed consistent patterns of differential gene expression across all ten strains associated with plasmid carriage. These findings shed light on the complex evolutionary dynamics between clinical plasmids and their bacterial hosts, with implications for understanding plasmid persistence, dissemination, and the evolution of antibiotic resistance in clinical settings.

P045

Within-patient evolution of ciprofloxacin resistance in Pseudomonas aeruginosa across a large-scale clinical trial

Matthew Shepherd[1], Niamh Harrington[2], Taoran Fu[1], Anastasia Kottara[3], Kendall Cagney[2], James Chalmers[4], Joanne Fothergill[2], Dylan Childs[5], Steve Paterson[2], Michael Brockhurst[1]

[1]University of Manchester, Manchester, United Kingdom. [2]University of Liverpool, Liverpool, United Kingdom. [3]Manchester, Manchester, United Kingdom. [4]University of Dundee, Dundee, United Kingdom. [5]University of Sheffield, Sheffield, United Kingdom

Abstract

The antimicrobial resistance (AMR) crisis threatens to endanger modern medicine within the next 20-30 years. A key part of the problem is the emergence of AMR within patients themselves, during courses of antibiotic therapy. Whilst within-patient AMR emergence is increasingly receiving attention in the literature, investigations commonly involve a small number of patients and study single-patient cases which may poorly represent the more general patient population. Here we report finding on the evolution of ciprofloxacin resistance by *Pseudomonas aeruginosa* across a large-scale clinical trial. This trial utilised inhaled liposomal ciprofloxacin as therapy for patients with the lung condition bronchiectasis, who suffered with *P. aeruginosa* infection. We isolated a total of ~25,000 independent bacterial colonies, prior to treatment and during the year-long the trial, measured the ciprofloxacin MIC and growth rates in KB broth for all isolates, and performed whole genome sequencing of ~4069 isolates. Across the trial, ciprofloxacin MICs increased during periods of treatment and decreased during treatment withdrawal, in a pattern suggestive of resistance fitness trade-offs operating within patients. We also find that distinct phenotypic adaptive trajectories are followed in different patient cases, indicating diverse evolutionary dynamics driving resistance emergence. We also characterise the genetic mechanisms driving the within-patient evolution of resistance and highlight that this occurs through at least 3 distinct mechanisms for *P. aeruginosa*. These findings allow us to start characterising the mechanisms through with AMR can emerge within-patients during treatment, and to ask questions on how best we could predict resistance emergence in the future.

P046

Bottom-up microbial community assembly in an evolving world

Milla Similä, Teppo Hiltunen, Shane Hogle

University of Turku, Turku, Finland

Abstract

A central question in microbial ecology is understanding how species diversity arises and is maintained in communities. Traditionally, this question has been addressed using “bottom-up” community assembly approaches, where species are assumed to sequentially fill a habitat until the community becomes fully occupied. The degree to which this process is deterministic (predictable) from species and their traits is a matter of debate. Some emphasize the importance of competitive species hierarchies and environmental filtering, while others highlight the significance of stochastic events and historical contingency. The rapid evolution of competitive traits during community assembly is one potentially significant source of stochasticity. The impact of rapid evolution on the predictability of sequential community assembly from species pairs remains unknown. Here, we investigate how the predictability of microbial community assembly changes as environmental and evolutionary complexity increases. We constructed all possible subcommunities of four bacterial species, exposing them to a gradient of antibiotic concentrations and incorporating strains with different evolutionary histories. By including both antibiotic-resistant and susceptible strains, we evaluated how past evolutionary trajectories shape community dynamics under environmental stress. Our findings suggest that the evolution of antibiotic resistance alters competitive hierarchies among bacterial species, such that coexistence outcomes are contingent upon each species’ evolutionary history and the environment in which they grow.

P047

Plasmid or chromosome: the spread of antibiotic resistance in strain-structured bacterial species

Martin Guillemet[1], Sonja Lehtinen[2]

[1]ETH Zurich, Zurich, Switzerland. [2]Université de Lausannene, Lausanne, Switzerland

Abstract

Genes conferring resistance are found carried on either plasmids or chromosomes. These two strategies present a fundamental evolutionary trade-off: plasmids offer high mobility through horizontal gene transfer (HGT) but are often costly and unstable, while chromosomal integration provides stability at the cost of near vertical-only transmission. While it is well studied that horizontal gene transfer is beneficial to spread between species, the impact of within-species diversity and in particular of the strain structure found in most pathogenic bacteria is less clear. Here we combine stochastic analytical modeling and simulations of bacterial populations consisting of multiple strains maintained by balancing selection, such as that resulting from serotype-specific immunity or metabolic niche differentiation. Contrary to deterministic models, this allows us to better monitor the transient dynamics of chromosome or plasmid-borne resistance, and their sequential spread between strains. We find that despite segregation loss and a higher fitness burden, plasmid-borne resistance can transiently reach a very high frequency in a species. Yet in the long term we predict that chromosome-borne resistance should still displace the plasmid. This is consistent with recent observations of the temporal trend of carriage frequency on chromosomes and plasmids. We also find that the time-scale of this displacement is dictated by the number of strains, and depends on whether resistance brings a constant benefit or if it plateaus at an intermediate frequency. This work highlights a major impact of within species diversity and strain structure on bacterial evolution.

P048

Strain-sharing between nearby households drives extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* gut colonisation in rural Malawi

Angus O'Ferrall[1], David Lally[2], Peter Makaula[2], Gladys Namacha[2], Sekeleghe Kayuni[2,1], Janelisa Musaya[2], Russell Stothard[1], Adam Roberts[1]

[1]LSTM, Liverpool, United Kingdom. [2]MLW, Blantrye, Malawi

Abstract

Extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli*, resistant to third-generation cephalosporins and penicillins, are prevalent in faecal and environmental samples across Malawi and contribute to increased mortality in clinical infection. However, the evolutionary dynamics and local transmission patterns of these bacteria in rural communities remain poorly understood due to limited genomic surveillance.

In July 2023, we collected faecal samples from 211 individuals in rural southern Malawi. 153 were followed up in July 2024. ESBL-producing *E. coli* were isolated via selective culture. Gut colonisation prevalence rose from 33.3% to 54.2% ($p < 0.001$). To investigate genomic drivers, isolates underwent Illumina paired-end sequencing.

The dominant ESBL gene was $bla_{\text{CTX-M-15}}$, followed by $bla_{\text{CTX-M-27}}$. These genes were distributed across a diverse range of *E. coli* sequence types in both years. Resistance genes for aminoglycosides, sulfonamides, tetracyclines, and trimethoprim were widespread and stable through time. However, the distribution of *E. coli* sequence types shifted significantly over time. Single nucleotide polymorphism (SNP)-based phylogenetic analysis revealed several clonal groups from various sequence types that increased in prevalence (ST38, ST58, ST13823). Integration of isolate genomics with household GPS coordinates showed spatial clustering consistent with short-range inter-household transmission.

These findings highlight the role of localised strain-sharing in the expansion of ESBL-producing *E. coli* gut colonisation and underscore the need for genomic surveillance in rural African settings, where treatment options are limited when such bacteria cause clinical infection. Understanding these transmission dynamics can inform targeted Water, Sanitation, and Hygiene (WASH) interventions to reduce the spread of antimicrobial resistance (AMR).

P049

Survival Beyond the Gut: Investigating Aerotolerance Evolution in Campylobacter jejuni

Shani Ali[1], Evangelos Mourkas[2], Aidan Taylor[3], Ben Pascoe[4], Samuel Sheppard[4], Tiffany Taylor[1]

[1]University of Bath, Bath, United Kingdom. [2]Uppsala University, Uppsala, Sweden. [3]University of Reading, Reading, United Kingdom. [4]University of Oxford, Oxford, United Kingdom

Abstract

Pathogens that rely on direct host-to-host transmission are often exposed hostile external environments before infecting a new host. For obligate host-adapted pathogens, this phase imposes stress that may select for mutations enhancing environmental resilience. However, the evolutionary and molecular basis of this adaptation remains poorly understood. We explored these dynamics by investigating the evolution of aerotolerance in *Campylobacter jejuni* (*C. jejuni*), a microaerophilic bacterium adapted to avian gastrointestinal tracts but frequently recovered from diverse, oxygen-rich environments like water, poultry processing lines, and dairy products.

To investigate how *C. jejuni* adapts to oxidative stress, we performed experimental evolution using four isolates from lab, cattle, and water sources. Each isolate was passaged in triplicate through 30 cycles of alternating 12-hour aerobic shaking culture and 12-hour microaerophilic recovery to simulate selection for oxygen tolerance. Parallel control lines were maintained under standard conditions to isolate lab adaptation effects.

Evolved lines demonstrated enhanced aerotolerance, with improved survival and recovery under oxidative stress. Whole-genome sequencing of ancestral, evolved, and control lines identified candidate genes potentially linked to oxygen tolerance and environmental adaptation.

Our study highlights *C. jejuni's* capacity to adapt to oxygen-rich environments, suggesting that oxidative stress tolerance may be under positive selection in non-host settings. Future functional studies, including gene knockouts, aim to elucidate the molecular mechanisms driving this adaptation. A deeper understanding of these pathways could inform new strategies to curb *C. jejuni* transmission and enhance food safety interventions.

P050

Plasmid and Prejudice: Studying the fitness impacts of multi-drug resistance megaplasmids on diverse strains of Pseudomonas aeruginosa

Prajwal Vishwanath Bharadwaj, Samriti Midha, Marcus Blagrove, Jo Fothergil, James Hall

University of Liverpool, Liverpool, United Kingdom

Abstract

Plasmids drive bacterial innovation and evolution by transferring genes that can become crucial in stressful environments. Plasmids can impose a burden, or 'fitness cost' on host bacteria, which can vary dramatically based on the genetic background of the plasmid and host, and act as a barrier to plasmid transmission and maintenance. Though studies have explored the host range and fitness effects of plasmids in Enterobacteriaceae, non-model plasmids such as the drug resistance megaplasmids of *Pseudomonas* remain relatively underexplored. Studying the patterns and sources of fitness costs can help identify features of *Pseudomonas* likely to be associated with drug resistance plasmid dissemination. We transferred members of a widespread emerging multi-drug resistance megaplasmid family to >60 diverse strains of the opportunistic pathogen *Pseudomonas aeruginosa* and assessed the effects on bacterial fitness. We show that the same plasmid can impose hugely varying fitness costs on related strains of the same species, and, in some cases, provides fitness benefits even in the absence of antibiotics. Our findings suggest a need to look beyond existing notions of AMR plasmids persisting only in the presence of antibiotics and focus on features of plasmid-host compatibility that promote or prevent plasmid persistence. Studying the Pseudomonas pangenome, we aim to predict features interacting with plasmids and dictating plasmid maintenance and consequently identifying microbiomes sensitive to high transmission loads of antimicrobial resistance plasmids, allowing for more informed therapeutic approaches.

P051

RM system diversity: Maintenance and fitness benefits

Joseph Westley[1], Tatiana Dimitriu[2,1], Andrew Matthews[1], Iolanda Lopes Domingues[1], Mark D. Szczelkun[3], Stineke van Houte[1], Edze R. Westra[1]

[1]Environment and Sustainability Institute, Faculty of Environment, Science and Economy, University of Exeter, Penryn, United Kingdom. [2]School of Biology, University of St Andrews, St Andrews, United Kingdom. [3]DNA-Protein Interactions Unit, School of Biochemistry, Faculty of Life Sciences, University of Bristol, Bristol, United Kingdom

Abstract

Prokaryotes have evolved a diverse arsenal of defence systems that protect them from parasites such as bacteriophages, with the most prolific being restriction modification (RM) systems. RM systems function by methylating self-DNA at a specific sequence, and cleaving DNA if an unmethylated copy of the sequence is recognised. However, bacteriophages can escape these systems by chance acquisition of the methylation signature upon infection, allowing all progeny to infect cells with the same RM system. Being readily overcome, it is unclear why the remarkable RM system diversity observed in nature is maintained.

We hypothesize that 1) population-level diversity of RM systems can be maintained through negative-frequency-dependent selection, as epigenetic phage mutants specialize on the most abundant host strain and 2) that the resulting population-level diversity in RM systems confers stronger protection against evolving phages, preventing the invasion of bacteria with alternative resistance strategies.

Utilising flow cytometry to accurately monitor relative cell densities, we test these hypotheses by evolving lytic bacteriophage, lambda *vir*, on *E. coli* strains with different RM systems. Our experiments provide novel evidence of both the predicted negative frequency dependence of RM systems, and the protective advantage of higher population level RM diversity. These findings offer insight valuable to the wider study of defence system ecology, including how their remarkable diversity can be maintained and selected for, and how this diversity can feedback into the coevolutionary dynamics of bacteria and their parasites.

P052

c-di-GMP–Mediated Epigenetic Memory Drives Adaptive Evolution in Pseudomonas aeruginosa

Sima Modiri[1], Matthias Preusse[1], Mohammad Roghanian[2], Mathias Müsken[3], Lothar Gröbe[4], Susanne Häussler[1,5,2,6]

[1]Department of Molecular Bacteriology, Helmholtz Centre for Infection Research, Braunschweig, Germany. [2]Department of Clinical Microbiology, Copenhagen University Hospital - Rigshospitalet, Copenhagen, Denmark. [3]Central Facility for Microscopy, Helmholtz Centre for Infection Research, Braunschweig, Germany. [4]Flow Cytometry and Cell Sorting Platform, Helmholtz Centre for Infection Research, Braunschweig, Germany. [5]Institute for Molecular Bacteriology, TWINCORE, Hannover, Germany. [6]Cluster of Excellence RESIST (EXC 2155), Hannover Medical School, Hannover, Germany

Abstract

Background – Understanding how pathogenic bacteria develop adaptive phenotypes is essential for combating infectious diseases. While genetic variation provides raw material for evolution, phenotypic heterogeneity can accelerate adaptation by generating subpopulations with novel traits.

Methods – We performed adaptive laboratory evolution of *Pseudomonas aeruginosa* through repeated passaging in LB medium. To track phenotypic transitions and associated gene expression changes, we employed RNA sequencing, fluorescence-activated cell sorting (FACS) with transcriptional reporters, motility assays, and scanning electron microscopy (SEM).

Results – Evolved populations of different *P. aeruginosa* strains showed a shorter lag phase, indicating enhanced fitness, accompanied by complete loss of swarming motility while retaining normal swimming. Whole-genome sequencing revealed no adaptive mutations, suggesting epigenetic mechanisms stabilized gene expression. RNA-seq identified significant upregulation of Type VI secretion system (T6SS) genes and downregulation of flagellar and chemotaxis-related genes. Crucially, a c-di-GMP null strain did not exhibit these adaptive changes, implicating c-di-GMP signaling in adaptive evolution. Single-cell analyses of the wild-type evolved strain revealed pronounced regulatory heterogeneity, with key regulators exhibiting bistable expression. Sorting cells based on these expression states reproduced evolved phenotypes, suggesting a stable, heritable, memory-like state. Evolved strains also exhibited increased cell lysis rates during the transition state, potentially indicative of programmed cell death benefiting the surviving community.

Conclusion – Our findings demonstrate that c-di-GMP–mediated signaling and single-cell heterogeneity drive adaptive phenotypes in *P. aeruginosa*, highlighting the importance of epigenetic memory in bacterial evolution. Further investigation is needed to understand the regulatory dynamics of c-di-GMP signaling and the mechanisms underlying memory-like state inheritance.

P053

Just Keep Swimming - The role of genetic background in gene regulatory network rewiring events.

Mitchell Reynolds, Tiffany Taylor

University of Bath, Bath, United Kingdom

Abstract

Gene regulatory networks exhibit remarkable adaptability, but the factors shaping their evolutionary rewiring remain poorly understood. Using flagellar motility rescue as a model, I investigate how differences in global regulatory network architecture and genomic context influence the predictability of rewiring pathways across diverse bacterial species. Previous work has shown that in *Pseudomonas fluorescens*, motility can be restored in the absence of the master regulator FleQ through the co-option of NtrC, a response regulator associated with nitrogen uptake. To explore the generality of this process, we engineered deletions of the flagellar master regulator in five bacterial species—*Pseudomonas aeruginosa*, *Caulobacter crescentus*, *Vibrio cholerae*, *Escherichia coli* and *Bordetella bronchiseptica*—representing a range of regulatory network architectures. Under strong selection for motility, *P. aeruginosa* demonstrated rapid and repeatable motility restoration, similar to observations in *P. fluorescens*, with evolved mutants exhibiting slow motility within 42 hours. However, genome sequencing of motile isolates revealed distinct rewiring pathways, highlighting how variations in network architecture shape adaptive trajectories. By integrating these results across species, this study aims to uncover the principles governing the evolution of regulatory networks, providing broader insights into their adaptability and evolutionary dynamics.

P054

Evolutionary repair of a broken mitotic entry switch

Maria Rosa Domingo-Sananes[1], Chantelle Endeley[1,2]

[1]Nottingham Trent University, Nottingham, United Kingdom. [2]University of Nottingham, Nottingham, United Kingdom

Abstract

Biological processes are often regulated by molecular networks that create system-level behaviours, such as switches and oscillators. Over the last few decades, we have gained significant insights into the function of these systems from both theoretical and experimental perspectives. However, we lack knowledge of how these systems originated and how they continue to evolve. We have taken an experimental evolution approach to analyse how fission yeast cells adapt to defects in the control of mitotic entry. In this organism, mitosis is triggered by activation of the Cdc2-Cdc13 complex due to the removal of Wee1-dependent phosphorylation of Cdc2 by the Cdc25 phosphatase. Positive feedback loops between Cdc2 and its regulators help to ensure a robust and abrupt entry into mitosis by creating a bistable switch. We evolved cells with defects in the switch in four different strains (loss of function for Wee1, Cdc25, Cdc2 and loss of feedback regulation for Cdc25) along with wild-type controls for >250 generations in minimal media at 30˚C. For most populations and strains, we observed fitness increases and changes in cell size, with cells shifting towards wild-type sizes. The most significant changes in fitness and cell size occurred in cells with defects in Cdc25. Interestingly, whole-genome sequencing revealed that cells with different defects in Cdc25 acquired distinct mutations. Specifically, cells with defective, temperature-sensitive Cdc25 acquired mutations in cell cycle regulators in the same control network (Cdc2 and Wee1), whereas cells with defects in Cdc25 feedback regulation mostly acquired mutations in regulatory subunits of the proteasome.

P055

Dynamics of plasmid multimers in bacterial adaptation

Jakub Malerczyk[1,2], Roderich Römhild[1]

[1]Institute of Science and Technology Austria, Klosterneuburg, Austria. [2]Max Planck School Matter to Life, Heidelberg, Germany

Abstract

Plasmid multimers – common-in-nature, covalently-bonded repeats of plasmid sequence – exhibit interesting evolutionary traits, connected to mutation rates, cargo expression, and mutant establishment rates. As multimerization was recently found to facilitate resistance to β-lactams, studying the molecular aspects of the phenomenon is of applied interest. We modified a previously developed reporter system for characterization of different mutation types (allowing the differentiation of copy number mutations from point mutations) and put it on a plasmid known to multimerize. With this setup, we track expression of the selection marker and the total plasmid-cargo with fluorescent proteins, and combine it with population-level information on plasmid multimerization and fitness in an *Escherichia coli* host. Specifically, we contrast the evolutionary trajectory of populations starting in mono- and dimeric multimerization states under stable or fluctuating selection regimes. We find that environments with a low-expression-demand differentiated the multimerization profile of the dimeric populations while the monomeric ones remain unchanged in a fluctuating environment. Populations with different multimerization state show different dynamics of selection marker expression, total cargo expression, and fitness in terms of growth rate and yield. Plasmid multimers may thus have greater adaptive functions for growth in temporally dynamic environments than currently appreciated. This direction of study might be of general interest to evolutionary microbiologists and, through association with antibiotic resistance, relevant for public health.

P056

Preliminary Insights into Microbial Community Dynamics of Nasal Staphylococcus aureus Carriage

Duncan Ng[1], Dinesh Aggarwal[1,2], Katherine L. Bellis[1,3], Asha Akram[1], Beth Blane[3], Catarina Ribeiro de Sousa[1], Joe Brennan[1], Roisin Boggan[1], Josef Wagner[1], Stephen Kaptoge[3], Joan A. Geoghegan[4], John Danesh[3], Sharon Peacock[3], Julian Parkhill[3], Ewan M. Harrison[1,3]

[1]Wellcome Sanger Institute, Hinxton, United Kingdom. [2]Imperial College, London, United Kingdom. [3]University of Cambridge, Cambridge, United Kingdom. [4]University of Birmingham, Birmingham, United Kingdom

Abstract

Approximately one-third of people carry Staphylococcus aureus in the nose. *S. aureus* carriage is influenced by a range of factors, including host genetics, lifestyle, and interactions with the nasal microbiota. While previous studies have examined the relationship between S. aureus carriage and the microbiota, most have been underpowered and based on 16S rRNA sequencing, limiting taxonomic and functional resolution.

To address this, we established the CARRIAGE study, comprising 20,000 healthy individuals sampled at three time points, one week apart. From this cohort, we generated shotgun metagenomic data for 5,389 samples at the first time point and 430 participants across all three time points. A stringent decontamination pipeline was applied to remove likely contaminants.

We used unsupervised clustering to define community state types (CSTs) within the nasal microbiota. The most influential taxa driving variation were *S. aureus*, *Dolosigranulum pigrum*, *Corynebacterium pseudodiphtheriticum*, and *Corynebacterium accolens*. Our results support earlier findings that intermittent carriers have microbiome profiles resembling either persistent carriers or non-carriers. Strain-level analysis revealed that CST is partly shaped by strain-level differences, with longitudinal data confirming the persistence of both CSTs and specific strains over time. Pangenome analyses of dominant strains are ongoing.

These results advance our understanding of the microbial and strain-level factors associated with S. aureus carriage in the human nose.

P057

How does horizontal gene transfer impact the evolution of cooperation in bacteria?

Anna Dewar[1], Laurence Belcher[1], Thomas Scott[1], Geoff Wild[2], Ashleigh Griffin[1], Melanie Ghoul[1], Stuart West[1]

[1]University of Oxford, Oxford, United Kingdom. [2]Western University, London, Canada

Abstract

Cooperative interactions play a key role in the growth and success of many bacteria. Bacteria produce and secrete a range of molecules that provide cooperative benefits to the local population of cells. Horizontal gene transfer has been suggested as a mechanism to stabilize cooperation in bacteria. A key prediction of this hypothesis is that genes for cooperation should be overrepresented on more mobile parts of the genome, such as plasmids compared with chromosomes. To test this, we used the genomics tool SOCfinder to identify genes for cooperation in 4648 genomes from 146 bacterial species. We used phylogeny-based statistical methods to control for non-independence of species, and also for any unevenness in the taxonomic distribution of studied species. Contrary to the prediction, we found that genes for cooperation were instead more likely to be carried on chromosomes. We also used theory to show that while horizontal gene transfer might facilitate the initial invasion of cooperation, it has little impact on its longer-term maintenance in bacterial populations. Overall, the vast majority of genes for cooperation are not located on plasmids, suggesting that the more general mechanism of kin selection is sufficient to explain the prevalence of cooperative interactions across bacteria.

P058

Assessing the effect of varying antibiotic concentrations on the intracellular transposition of mobile genetic elements in *Escherichia coli*

Richard Goodman[1], Andrew Singer[2], Mike Brouwer[3], Peter Nambala[4,1], Nicholas Feasey[5,4,1], Nina Langeland[6], Sabrina Moyo[6,1], Adam Roberts[1]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom. [2]UK Centre for Ecology & Hydrology (UKCEH), Wallingford, United Kingdom. [3]Wageningen University, Lelystad, Netherlands. [4]Malawi Liverpool Wellcome Clinical Research Programme, Blantyre, Malawi. [5]University of St Andrews, St Andrews, United Kingdom. [6]University of Bergen, Bergen, Norway

Abstract

Antimicrobial resistance (AMR) is a major threat to human health. Inter- and intracellular gene transfer drives the emergence and persistence of AMR in Enterobacterales. The use of antimicrobial compounds in human, animal and agricultural contexts leads to environmental contamination through wastewater. The effects of these compounds on intracellular transposition within environmental isolates have not been previously assessed. Here we introduce two *E. coli* strains, each containing a fully sequenced and annotated chromosome, plasmid and entrapment vector (a triple replicon system). The only difference between the two replicon systems is the selectable marker in the entrapment vector, carrying either a colistin or kanamycin resistance gene. We assess the effect of differing concentrations (0ng/L, 32ng/L, 320ng/L and 3200ng/L) of the third-generation cephalosporin ceftriaxone on the rate of transposition between replicons within the host. We show that transposition occurs at low rates, but certain concentrations have a significant effect on the number of transposition events detected. The lowest observed effect concentration (LOEC) for ceftriaxone was found to be 320 ng/L, and the no observed effect concentration (NOEC) was 32 ng/L. This provides a minimum threshold for the environmental impact of ceftriaxone on biological systems at the sub-cellular scale, which is applicable to industrial standards of waste management, where consideration of ecological impact is central. Overall, we demonstrate how sub-inhibitory concentrations of an antibiotic can influence intracellular transposition. Furthermore, our triple replicon system provides an adaptable platform for investigating the impact of various environmental stressors on mobile element and associated AMR dynamics.

P059

Environment-dependent phage evolution destabilizes the *Salmonella*-phage coexistence mediated by phase variation

Nicolas Wenner, Anouk Bertola, Leonardo Lemos Rocha, Médéric Diard

Biozentrum, University of Basel, Basel, Switzerland

Abstract

Heterogeneous gene expression enables the coexistence of virulent phages with populations of isogenic bacteria comprising both phenotypically phage-resistant and susceptible cells. However, phage evolution may challenge the stability of the coexistence. To study these complex dynamics, we built on the discovery that the bistable alteration of the O-antigen (phase variation) enable phenotypic resistance in *Salmonella*Typhimurium (*S*.Tm) against the T5-like phage Φ37. This bistable phenotypic resistance mechanism allows long term phage-bacteria coexistence in LB medium under classical laboratory conditions. In contrast, by tracking the coevolution of *S*.Tm and Φ37 in the intestine of infected mice, we found that phase variation only delays the fixation of genetically phage-resistant *S*.Tm mutants and coexistence is short-lived in the gut. This destabilization is caused by the emergence of evolved phages capable of infecting both phenotypically susceptible and resistant phase variants of wild-type *S*.Tm, causing the selection of genetically resistant *S*.Tm mutants. The fact that *S.*Tm and Φ37 can coexist in LB but not in the gut, suggested that environmental parameters influence the evolutionary fate of Φ37 in response to *S*.Tm O-antigen phase variation. We found that increasing the viscosity of a medium using the polymer PVP mimics and recapitulates the short-lived coexistence observed in the gut. This work reveals for the first time how environmental conditions modulate phage evolution in response to phenotypic resistance.

P060

Assessing defence phenotypes when varying expression levels of *Pseudomonas aeruginosa* antiphage defence systems.

Meg Llewellyn, Aleksei Agapov, Anna Olina, Stineke van Houte, Edze Westra

University of Exeter, Penryn, United Kingdom

Abstract

Bacteriophages are viruses that can infect, kill and alter bacteria. Existing in a 5-10-fold excess to bacteria, phages create a strong selection pressure for bacteria and subsequently have driven the evolution of an arsenal of anti-phage defence systems (DSs). Researchers at the Westra lab previously modified *Pseudomonas aeruginosa* strain PAO1 by removing all known anti-phage DSs and introduced individual DS plasmids to assess their function against a panel of diverse phages. This revealed an unexpected, extensive lack of protection, leading to the question of whether the DSs are functional, and what conditions may affect the protection levels they provide. To test this, we have replaced the DSs native promoters with inducible promoters to allow us to see how overexpression affects the system's defence phenotypes. We saw, predominantly, a large increase in costs for most defence systems when overexpressed. However, some systems' protection levels increased. We hope to broaden the understanding of the functionality and protection levels of some of the most abundant *Pseudomonas aeruginosa* defence systems.

P061

Diversity and Evolution of *Pseudomonas syringae* Population in Nature

Xiaoyi Hu[1], Pauline W. Wang[1,2], Sumer Horuz[3], Yesim Aysan[4], David S. Guttman[1,2]

[1]Department of Cell and Systems Biology, University of Toronto, Toronto, Canada. [2]Centre for the Analysis of Genome Evolution and Function, University of Toronto, Toronto, Canada. [3]Department of Plant Protection, Erciyes University, Kayseri, Turkey. [4]Department of Plant Protection, University of Çukurova, Adana, Turkey

Abstract

The phytopathogenic bacterium *Pseudomonas syringae* is a highly diverse, globally distributed species complex that causes disease in a wide range of plant hosts. While excellent work has already been performed on the comparative and evolutionary genomics of *P. syringae*, most studies have focused on strains isolated from specific host species or disease outbreaks, often relying on single-isolate sampling from single host plant, region, or host species. The diversity and population dynamics of *P. syringae* across different ecological strata remain poorly characterized. Here, we analyzed a collection of *P. syringae* strains sampled hierarchically across multiple ecological strata in Turkey, including individual disease lesions, leaves, host plants, greenhouses, towns, and provinces. From an initial set of 516 samples subjected to whole-genome sequencing, we generated 448 high-quality draft genomes for downstream analysis. Using an integrated analytical approach combining phylogenetic construction, pangenome analysis, Bayesian population structure inference, and fine-scale genetic variation profiling, we systematically characterized the core and accessory genome architecture, pangenome dynamics, and population structure. Our preliminary results demonstrate substantial genetic diversity both within and between ecological strata, with variance partitioning revealing differential contributions from different hierarchical levels. We identified distinct population clusters associated with specific strata, while ongoing pangenome analyses are characterizing both conserved and niche-specific genomic features. This unique dataset provides a valuable resource for understanding the ecological and evolutionary drivers of *P. syringae* population dynamics, offering new insights into pathogen emergence, transmission, and adaptation across spatial and host-associated ecological strata.

P062

Coalescence dynamics in hot spring microbial communities

Magdalena Kurteu, Katharine Coyte, Sophie Nixon, Michael Brockhurst

The University of Manchester, Manchester, United Kingdom

Abstract

Microbial communities support the functioning of a diverse range of ecosystems, from soil to rivers, food production to human digestion. These communities are dynamic and constantly evolving systems, influenced by environmental changes as well as frequent mixing with other communities in a process called community coalescence.

We are investigating CO_2 fixing microbial communities from Icelandic volcanic hot springs with the ultimate aim of harnessing their ability to metabolise industrial waste gasses.

Here we show an experimental community coalescence approach via culture directly from hot spring samples. Highlighting the dynamic nature of these community systems and exploring implications and future direction of this approach.

P063

Genomic insights into *Klebsiella pneumoniae* wastewater isolates harbouring carbapenem resistance in Malawi

Ellinor Shore[1], Peter Nambala[1], Nina Langeland[2], Nicholas Feasey[3,1,4], Sabrina Moyo[1], Adam Roberts[1]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom. [2]University of Bergen, Bergen, Norway. [3]Malawi Liverpool Wellcome Programme - Kamuzu University of Health Sciences, Blantyre, Malawi. [4]School of Medicine - University of St Andrews, St Andrews, United Kingdom

Abstract

The global dissemination of carbapenem-resistant *Enterobacterales* (CRE) is a major public health concern. Carbapenemase enzymes confer resistance to multiple antibiotic classes, rendering many treatment options ineffective. In this study, we employed whole genome sequencing (WGS) to investigate the population structure and genomic context of antimicrobial resistance genes (ARGs) in Klebsiella pneumoniae (n=139) isolated from wastewater at Queen Elizabeth Central Hospital (QECH), in Blantyre, Malawi, between May 2023 and May 2024. All isolates harboured at least 2 ARGs predicted to confer resistance to beta-lactams, with 97.1% (135/139) carrying *bla*CTX-M-15. Carbapenemase genes were detected in 20.1% (28/139) of isolates, including blaNDM-1 (10.7%, 3/28), and *bla*NDM-5 (89.3%, 25/28). To our knowledge, this marks the first identification of blaNDM-1 in Malawi, and the first identification of *bla*NDM-5 in a K. pneumoniae isolate from Malawi. Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) isolates were of sequence type (ST), ST147 (28.6%, 8/28), its sublineage ST1880 (67.8%, 19/28), or ST34 (3.6%, 1/28). With the exception of the ST34 isolate, all blaNDM-5 isolates exhibited shared resistance patterns, high average nucleotide identity ≥99.93%, and ≤12 single nucleotide polymorphisms (SNPSs), indicating a clonal population persisting in the wastewater system for 8-months. These findings address a knowledge gap in microbial surveillance and molecular epidemiology of CRKP between One Health sectors in Malawi and sub-Saharan Africa, emphasising the need for coordinated surveillance within and between these different sectors.

P064

Understanding the adaptive rewiring of evolved respiratory variants of *Escherichia coli* using multi-scale computational biology approaches

Arjun Patel[1], NEHA BANWANI[2], Bernhard O. Palsson[1,3], Amitesh Anand[2]

[1]University of California, San Diego, USA. [2]Tata Institute of Fundamental Research, Mumbai, India. [3]Technical University of Denmark, Denmark, Denmark

Abstract

Bacteria thrive in complex environments that require them to have a robust metabolism, often dependent on an adaptable energy metabolism (Srivastav S. *et al*, 2024, J Biol Chem). In this systems level study, optimization approaches rendered by E. coli MG1655 bioenergetic variants, each having an unbranched Electron Transport System (ETS) which pumps 1, 2, 3, 4 protons per electron (ETS-1H, 2H, 3H and 4H respectively), was investigated (Anand A. *et al*, 2022, Nat Commun).

These bioenergetic variants were evolved in the presence of metabolically restrictive carbon sources like glycerol, which supports mixed glycolytic and gluconeogenic fluxes, and succinate, which requires complete gluconeogenesis. Upon adaptive laboratory evolution, followed by whole genome sequencing of the evolved bioenergetic variants, glycerol retention and succinate transport related mutations in their respective media were observed. A quantitative descriptor called "Aerotype", an indicator of aerobicity of the cell (Chen K. *et al*, 2021, PLoS Comput. Biol.) was also studied, with ETS-2H and ETS-4H showing higher aerobicity, suggesting its link to specific ETS complexes. Computational models employing metabolite exchange rates, growth rates and transcriptomics data as constraints for metabolism and expression (ME) modelling were used, exhibiting preferences of ETS complex usages and their aerobicities, similar to our previous glucose study (Anand A. *et al*, 2022, Nat Commun). Further, using iModulon analysis, a novel aerobicity stimulon was identified, exhibiting substrate-independence. Our study provides a fundamental understanding of bacterial bioenergetics and its link with cellular metabolism, hence enhancing our knowledge about bacterial physiology and its plasticity.

P065

Bacteriophage T7 evolution with phage-resistant *E. coli* LPS mutants

Piotr Burzynski[1], Maisem Laabei[2], Wolfram Moebius[1]

[1]University of Exeter, Exeter, United Kingdom. [2]University of Bristol, Bristol, United Kingdom

Abstract

Bacteriophages depend on specific features of the outside of a bacterial cell to adsorb to and infect their host. It is therefore not surprising that modifications of those structures can reduce phage adsorption and thus infection, triggering phage evolution to overcome this resistance. For example, the importance of lipopolysaccharides (LPS) for bacteriophage T7 adsorption is well established, as is the potential for T7 to overcome this resistance. However, we are missing a systematic picture of bacteriophages' evolutionary response to changes in the LPS.

We evolved bacteriophage T7 on several strains of *E. coli* with knockouts in LPS biosynthesis genes (Δ*waaR*, Δ*waaG*, Δ*gmhA*, Δ*gmhD* and Δ*waaC*) using the classical top agar assay. Isolating phage from individual plaques revealed that phages evolved on hosts with knockouts of genes that interrupt early stages of the LPS synthesis, such as *E. coli* Δ*waaC*, were consistently able to cross-infect most, if not all, other LPS mutants investigated. Phage evolved on *E. coli* Δ*waaR* and Δ*waaG* had a reduced, but more variable, capacity to infect strains with a further reduced LPS. Further evolving phage on a different host, for example, evolution on *E. coli* Δ*waaC* following evolution on *E. coli* Δ*waaR,* highlights the path-dependence of phage evolution even in response to these relatively similar hosts.

In addition, we are complementing this phenotypic characterisation by genotypic characterisation, with preliminary sequencing results indicating the importance of mutations in the genes coding for tail fibres in bacteriophage host recognition.

P066

Differences in resistance evolution and genetic mechanisms between closely related antimicrobial peptide sequences

Sophie Somerville, Curtis Dobson, Danna Gifford, Chris Knight

University of Manchester, Manchester, United Kingdom

Abstract

Combatting the global rise of antimicrobial resistance requires the development of new therapeutic antimicrobials and a greater understanding of how resistance evolves. Antimicrobial peptides (AMPs) are considered a promising area for the development of new therapeutics, partly due to their reduced propensity for resistance to evolve against them compared to other antibiotic classes. Despite this, resistance has been observed against clinically used AMPs such as colistin. Much is known about how peptide sequence affects characteristics such as antimicrobial activity, stability and toxicity, down to understanding the effect of individual amino acid changes. In comparison, little is known about how sequence affects the evolution of resistance to AMPs with closely related sequences.

Using experimental evolution with five heptapeptides containing exclusively arginine and tryptophan residues, we investigate the frequency and genetic mechanisms of resistance evolution in *Escherichia coli*. Despite being closely related in sequence, we see differences among peptides in the frequency and extent of resistance evolving. Cross-resistance between peptides was common, though varied between peptides tested. Whole genome sequencing of peptide-resistant strains revealed a variety of potential resistance mechanisms. Some mechanisms, such as alterations to genes in the outer membrane integrity maintenance pathway (Mla), occur only in strains evolved with specific peptides. Others, such as loss-of-function of the Skp outer membrane protein chaperone, evolve in response to almost all peptides tested. We conclude that sequence differences between our peptides impact the likelihood of resistance evolving, and that genetic mechanisms of resistance to closely related peptides can be either generic or specific.

P067

The evolution of bacterial interactions in a synthetic *Salmonella* - *Escherichia coli* Nissle community

Fran Gilis, Bram Lories, Hans Steenackers

KU Leuven, Leuven, Belgium

Abstract

Bacterial communities are shaped by complex interaction networks, yet the evolutionary transition from competition to cooperation remains poorly understood. One proposed mechanism suggests that a cooperative interaction can evolve from an initially accidental positive effect of one strain on the other. To explore the ecological conditions that support this transition, we are combining experimental and computational approaches using a synthetic community composed of *Salmonella* and *Escherichia coli* Nissle.

In our experimental system, we created synthetic *Salmonella* – *E. coli* Nissle communities that vary in the initial level of competition and simulate the emergence of an accidental positive effect by introducing an environmental detoxification mechanism in one of the two strains. These communities were monitored for short-term stability using serial transfer experiments. In parallel, we developed a population-level model to simulate the dynamics of the two-species communities. This model enables to predict the long-term dynamics of the two-species communities and will be extended with an evolutionary compartment to explore the evolution of the bacterial interactions starting from a wider range of initial conditions.

The screening of the synthetic communities for short-term stability resulted in the selection of co-existing configurations to be subjected to long-term evolution experiments assessing how bacterial interactions evolve under different ecological conditions. Furthermore, preliminary efforts were made in predicting long-term stability using the computational model.

In conclusion, this integrative approach aims to elucidate the ecological conditions that support the evolution of cooperative interactions from accidental effects, advancing our ability to rationally design microbial communities with predictable evolutionary trajectories.

P068

Mapping the Network of Crosstalk between Arbitrium Phages

Rebecca Woodhams[1], Robyn Manley[2], Edze Westra[1]

[1]University of Exeter, Penryn, United Kingdom. [2]University of Exeter, Exeter, United Kingdom

Abstract

Bacteriophages are the most abundant biological entities on the planet and their infection dynamics shape microbial communities. Temperate phages choose between two lifecycles upon infection; lytic, where they lyse their host to release new virions, or lysogenic where they integrate into the bacterial genome. SpBeta-like phages utilise a communication system similar to quorum sensing to coordinate this decision upon infection of their *Bacillus* host. The concentration of so-called Arbitrium signal molecules indicates the availability of susceptible host cells, enabling the infecting phage to make an appropriate lifecycle decision.

Closely related phages produce different signal molecules. Canonically, it was believed that phages could only sense their own signal. However, we demonstrate that the infection dynamics of Phi3T (an SpBeta-like phage) are affected by non-cognate signals, a phenomenon we call “crosstalk”. Using a collection of both natural and chimeric Phi3T phages, we establish the communication network between these phages. We confirm that phages carrying arbitrium systems that employ the same signal peptides also show similar patterns of cross talk, even if the phages themselves have overall limited sequence similarity. We find that cross talk is common but limited to related arbitrium systems, cross talk can be either bidirectional or unidirectional between systems, and systems vary in their promiscuity. Overall, we show that naturally occurring phage-derived arbitrium peptides can manipulate the lysis-lysogeny switch of distantly related phages that carry similar arbitrium systems.

P069

Global phylogeographic analysis of the emergence and spread of AMR typhoid

Zoe Dyson[1,2], Danielle Ingle[3], Gaetan Thilliez[4], Megan Carey[1], Philip Ashton[5,6], Yogesh Hooda[7], Satheesh Nair[8], Sophie Octavia[9], David Rasko[10], Michael Sikorski[10], Kathryn Holt[1,11], Global Typhoid Genomics Consortium[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom. [2]Wellcome Sanger Institute, Hinxton, United Kingdom. [3]The University of Melbourne, Melbourne, Australia. [4]Quadram Institute Bioscience, Norwich, United Kingdom. [5]Malawi-Liverpool Wellcome Programme, Blantyre, Malawi. [6]University of Liverpool, Liverpool, United Kingdom. [7]Child Health Research Foundation, Dhaka, Bangladesh. [8]United Kingdom Health Security Agency, London, United Kingdom. [9]University of New South Wales, Sydney, Australia. [10]University of Maryland, Baltimore, USA. [11]Monash University, Melbourne, Australia

Abstract

Background: Typhoid fever is a faeco-orally transmitted systemic infection caused by *Salmonella* Typhi. Each year >10 million cases occur worldwide of which >100,000 are associated with mortalities. Antimicrobial chemotherapy has been the mainstay of typhoid control, but is increasingly threatened by antimicrobial resistance (AMR).

Methods: The Global Typhoid Genomics Consortium recently reported on the global diversity and AMR patterns of Typhi in a meta-analysis of >13,000 genomes. Here, we carried out dated phylogeographical analyses of those data to better understand the spatiotemporal origins and spread of AMR variants.

Results: In Asian and African regions where typhoid is endemic, the burden of resistant infections is mostly associated with local transmission of resistant variants (66-99% of cases). Resistance to classical first line drugs chloramphenicol, trimethoprim-sulfamethoxazole, and ampicillin (multidrug-resistance; MDR) emerged on at least 126 occasions, from the 1980s onwards in Southern Asia. Most fluoroquinolone resistance emergence events occurred from the 1980s, becoming fixed on at least 125 occasions. Persistent co-circulation of multiple distinct ciprofloxacin non-susceptible (CipNS) variants occurred in most endemic settings, with the greatest diversity occurring in Bangladesh and India (n=8-33 unique variants per year; mean annual Simpson's diversity >0.8). The proportion of onward-transmitted CipNS mutations per country was associated with mean national annual consumption of quinolones (R^2=0.42, p=0.018, linear regression).

Conclusions: Our findings suggest that improvements to water sanitation and hygiene (WaSH) infrastructure and the use of typhoid conjugate vaccines should be prioritised to disrupt AMR typhoid transmission in endemic settings.

P070

Microbial warfare: a driver of antimicrobial resistance?

William Smith, Michael Brockhurst

University of Manchester, Manchester, United Kingdom

Abstract

Microbes living in mixed communities are often exposed to deadly toxins produced by competing strains. These include the diverse protein toxins secreted by Type 6 Secretion Systems (T6SSs), a widespread mediator of intermicrobial warfare. Our recent work has shown that bacteria can evolve resistance to T6SS toxins via multiple mechanisms, but the broader effects of these adaptations on antibiotic susceptibility are poorly understood. To address this knowledge gap, we combined high-throughput MIC screening with bioinformatic analyses to test whether adaptations to T6SS aggression can alter antibiotic susceptibility. Using strains both from the literature and our own collections, we assembled a library of 48 T6SS-resistant *E. coli* strains, and used an automated broth dilution screen to profile strains' sensitivities to a diverse panel of antibiotics, antimicrobial peptides and detergents. We found that multiple T6SS resistance genotypes led to cross-resistance to some antimicrobials, while generating collateral sensitivity to others. Significantly, we observed that mechanisms associated with broad-spectrum T6SS resistance tended to also grant cross resistance to polymyxin, tetracycline and fluoroquinolone antibiotics, but led to collateral sensitivity to nitrofuran and detergent antimicrobials. We conclude that bacterial adaptation to T6SS-mediated competition can collaterally alter antimicrobial resistance, suggesting that competitive communities may select for predictable changes in antimicrobial sensitivity profiles. By revealing new ecological and evolutionary pressures that maintain AMR genes within microbial communities, our work helps to tackle the proliferation of resistance in hosts and environmental reservoirs.

P071

Animal-microbe symbioses in pest insects

Katie Millar

University of East Anglia, Norwich, United Kingdom

Abstract

The Mediterranean fruit fly (*Ceratitis capitata*) is a significant agricultural pest, known for its widespread impact, infesting up to 300 species of fruits and vegetables. It has been extensively studied as a target for biocontrol, and the role of its microbiome in shaping its biology remains an emerging area of ongoing research. Closely related fruit flies, such as the olive fruit fly (Bactrocera oleae), are known to rely on obligate symbiotic relationships with gut bacteria for survival. However, while the Mediterranean fruit fly does not exhibit such specialised dependencies, it has nonetheless been consistently found to have a microbiome dominated by a single bacterial species, *Klebsiella oxytoca,* regardless of factors like diet, geographical location or population genetics. This persistent presence of Klebsiella suggests a potentially important role for this bacterium in the fly's physiology, nutrition and ecological interactions. Understanding the dynamics of the Medfly microbiome, including the role of *Klebsiella* and other microbes, could provide valuable insights into the host's adaptability, development, and potential vulnerabilities. In my PhD research, I am aiming to study the microbiome of the Mediterranean fruit fly to reveal novel targets for pest control strategies. Through this, microbiome manipulation can be leveraged as an innovative approach to managing this major agricultural pest.

P072

Understanding the functional and genetic diversity of a collection of lytic bacteriophages for screening against Pseudomonas aeruginosa clinical isolates

Anna Richmond[1], Rama Bhatia[1], Mark Szczelkun[2], Stineke van Houte[1]

[1]University of Exeter, Penryn, United Kingdom. [2]University of Bristol, Bristol, United Kingdom

Abstract

Bacteriophages (phages) are viruses that specifically target bacteria, they are commonly highly host-specific and represent an alternative to antibiotics. Bacteria carry defence systems against phages. These defence systems can evolve to provide resistance against specific phages. The importance of such systems is not currently fully understood.

We generated a collection of over 80 lytic phages that target the multi-drug resistant *Pseudomonas aeruginosa*. These phages were analysed for their taxonomic, functional, genetic and proteomic diversity. Revealing a diverse phage panel containing an 8-fold difference in genome size, multiple anti-defence systems and different receptors for cell entry. This analysis covers phage entry into cells, phage metabolism, and genetic similarity, all potential targets for bacterial defence systems.

This phage collection will be screened for infectivity against a diverse range of 300 *P. aeruginosa* clinical isolates. These clinical isolates originate from hospitals across Europe and the Middle East and have been sequenced and analysed for defence systems. They carry between 4 and 29 potential defence systems per genome.

Therefore, this high-throughput screening will establish the effectivity of a range of phage profiles against different defence systems in their natural host. This work is part of the sLoLa Multi-Defence project and will further the understanding of the relationship between defence systems and phage resistance, which will be crucial in establishing successful phage therapy for *P. aeruginosa* infections.

P073

Understanding the impact of horizontal gene transfer on multi-layered defence systems

Shreya Vichare, James Hall

University of Liverpoool, Liverpool, United Kingdom

Abstract

Bacteria frequently encounter mobile genetic elements (MGEs) such as plasmids, phages, and transposons. The acquisition of these MGEs through horizontal gene transfer (HGT) can enable bacterial genomes to adapt to new environments, promoting niche expansion. Such an acquisition of MGEs may impose fitness costs, thus driving the evolution of a variety of defence mechanisms in bacteria to protect their genomes from MGE invasions. Interestingly, multiple mechanistically distinct defence systems can coexist within a single bacterial genome. This has led to the hypothesis that these systems may function cooperatively, forming multi-layered defence network that acts at different stages to counter specific MGE attacks. Surprisingly, recent studies have revealed that many of these defence systems are themselves encoded on MGEs. The evolutionary drivers of this localization — and its influence on the MGE's own horizontal transfer potential — remains vastly understudied. In this project we will explore the effect of mobility on the success of defence systems by expressing different defence systems from a conjugative plasmid in *Pseudomonas aeruginosa* PAO1. This approach will allow us to examine how horizontal gene transfer influences multilayered defence strategies and to assess the propensity of HGT of individual defence systems. Our study aims to elucidate how the mobility of genome defence systems shapes the selection for defence coalitions in the presence and absence of a threat such as phage infection or, an opportunity such as a MGE borne trait. Consequently, we hope to advance the understanding of the ecology, evolution, and function of mobile, multilayered bacterial genome defences.

P074

Species background influences evolution of antimicrobial peptide resistance in the *Enterobacteriaceae*

Sarah Duxbury[1], Josh Hodges[1], Elizabeth Constable[1], Michael Jardine[1], Thomas Price[1], David Studholme[2], Ben Raymond[1], Nick Royle[1]

[1]University of Exeter, Penryn, United Kingdom. [2]University of Exeter, Exeter, United Kingdom

Abstract

Antibiotic resistance presents a substantial threat to human and animal health due to decreased antibiotic efficacy. Antimicrobial peptides (AMPs)- immune effectors- present promising alternatives, due to their coevolution with microbial pathogens and competitors. Despite AMP susceptibility across bacterial pathogens and predicted lower propensity for resistance evolution compared with antibiotics, the drivers and constraints of AMP resistance evolution remain poorly understood. Here we investigated dynamics of evolution in two key opportunistic pathogens of the *Citrobacter* and *Enterobacter* genera, against AMPs of diverse origin and predicted physicochemical properties: insect (cecropins), bacterial (polymyxin B) and human (LL-37) origin. Based on prior studies, we hypothesised that AMP adaptation would occur with the greatest MIC increases for polymyxin B, followed by LL-37 and the cecropins. Replicate populations of each species were evolved under stepwise two-fold AMP dose increases, imposing strong selection. Passaging of parallel populations without AMP allowed to distinguish medium adaptation. Results indicate that both species adapted to grow at significantly greater AMP concentrations (up to 10-fold and higher) with increased MICs. Adaptation against polymyxin B was lower, whereas higher-level resistance developed in the *Enterobacter* species against one of the cecropins. Ongoing experiments are measuring growth fitness, cross-resistance against AMPs and antibiotics and performing sequencing to determine resistance mechanisms. Our results support AMP resistance evolution in the *Enterobacteriaceae*, dependent upon both AMP and species background. These findings highlight the importance of testing AMP efficacy across a range of species, combined with fitness effects, to allow predictions of longer-term AMP efficacy in mixed-strain contexts.

P075

Culturomics insights into phenotypic antimicrobial resistance evolution in the gut microbiota of allogeneic stem cell transplant patients

Elisa Sosa[1], Ana Gabriela Monsalve[1], Klara Haldimann[1], Diana Albertos Torres[1], Julia D. Schulze[2], Simon M. Mueller[2], Joerg Halter[3], Adrian Egli[1]

[1]Institute of Medical Microbiology, University of Zurich, Zurich, Switzerland. [2]Department of Dermatology, University Hospital Basel, Basel, Switzerland. [3]Department of Hematology, University Hospital Basel, Basel, Switzerland

Abstract

Allogeneic stem cell transplant (allo-HSCT) recipients are at high risk for bacterial infections and receive frequent antibiotics, driving antimicrobial resistance (AMR). We aim to explore the prevalence and resistance of gut bacteria at single-strain resolution.

Weekly stool samples from 100 patients from pre-transplant to six months post-transplant were collected and streaked on agar plates. Twenty random colonies per sample were isolated, identified by MALDI-TOF and biobanked. Phenotypic AMR of *Escherichia coli* was assessed by EUCAST disc diffusion with amikacin, ceftriaxone, levofloxacin, cefepime, piperacillin/tazobactam, and amoxicillin/clavulanate.

Preliminary data from eight patients included 9'756 isolates representing 73 species, most frequently *E. coli* (42.7%), *Enterococcus faecalis* (20.2%), and *E. faecium* (17.3%). We observed up to 9 unique species per agar plate, with monoculture growth in 23.5% of non-selective agar. In patient 06, diversity recovered to 6 species within one week, highlighting the impact of interspecies dominance and selection pressure on community structure.

Among 695 *E. coli* isolates, 4'193 inhibition zone measurements revealed shifts in susceptibility. In patient 06, the median levofloxacin inhibition zone dropped from 31 mm (sensitive; interquartile range [IQR] 31.0–31.5mm) to 6mm (resistant; IQR 6.0–6.0mm) after one week. In patient 04, both resistant and sensitive populations were measured at 17 out of 28 timepoints against amoxicillin/clavulanate, indicating heterogeneity.

This study provides insights into AMR evolution in gut bacteria during allo-HSCT, highlighting strain-level adaptations to antibiotic pressure and the potential of culturomics to reveal resistance dynamics. As the study progresses, expanded sampling and sequencing will be performed.

P076

The presence of a secondary species alters P. aeruginosa ciprofloxacin resistance evolution in the cystic fibrosis lung.

Sadhbh Dodd, Siobhán O'Brien

Trinity College Dublin, Dublin, Ireland

Abstract

The cystic fibrosis (CF) lung is a harsh and heterogenous environment, and can impose selection and stress on the microbial community. The varying concentrations of antibiotics that are administered to target specific community members cannot permeate completely into the environment due to the complex lung structure, and results in sub-inhibitory concentrations of antimicrobials being present in the environment, affecting key members of the CF community, including *S. aureus* and *P. aeruginosa*.

While it is clear that sub-inhibitory concentrations of antibiotics can drive antimicrobial resistance (AMR) in simple single-species lab studies, it is less clear if or how AMR evolution will proceed in the context of a microbial community. To understand how the presence of both a secondary species and sub-inhibitory concentrations of antibiotics impact the *de novo* mutation rate and antibiotic resistance evolution of *P. aeruginosa*, we performed a 20-day evolution experiment of *P. aeruginosa* and *S. aureus* exposed to sub-inhibitory concentrations of ciprofloxacin, a commonly prescribed fluoroquinolone antimicrobial.

We show that in both single- and dual- species environments, *P. aeruginosa* rapidly evolves ciprofloxacin resistance via mutations in DNA gyrase components and overexpression of multidrug resistance pumps. However, the route to resistance of *P. aeruginosa* differs depending on the presence of *S. aureus*. *P. aeruginosa* populations evolved alone acquired mutations in *gyrB*, and *P. aeruginosa* populations evolved with *S. aureus* acquired mutations in *nfxB*. Our work highlights the importance of incorporating a community when investigating antimicrobial resistance in CF, and how evolutionary trajectories can be determined by community presence.

P077

RecA deletion prevents the evolution of effective nitrofurantoin resistance.

Laura Domínguez Mercado, Brandon Findlay

Concordia University, Montreal, Canada

Abstract

Antibiotic resistance presents a growing threat to public health, killing millions every year. Notably, nitrofurantoin remains relevant in clinical settings. A key bacterial stress response to nitrofurantoin is the SOS pathway, triggered by DNA damage. RecA regulates this response and promotes DNA repair and mutagenesis. RecA-deficient strains show up to 64-fold increased sensitivity to nitrofurantoin, suggesting a strong link between the SOS response and resistance development.

This study investigates RecA's role in nitrofurantoin resistance and evaluates RecA inhibition as a therapeutic strategy. Using the Soft Agar Gradient Evolution (SAGE) system, we tracked resistance in 32 independent lineages of *E. coli* BW25113 and *E. coli* Δ*recA*. This system provides a continuous concentration gradient, where bacteria swarm through and accumulate resistance mutations. We also compared genetic and chemical SOS inhibition. Three reported RecA inhibitors were tested for their ability to reduce nitrofurantoin resistance.

We found that Δ*recA* strains evolved significantly lower resistance levels; up to 1024-fold less than wild-type. In contrast, chemical inhibition of RecA failed to reproduce the full hypersensitivity seen in genetic knockouts. We characterized the evolutionary trajectory of nitrofurantoin resistance independent from the SOS response using WGS and identified the mutations leading to resistance, as well as lineages that failed to evolve.

These findings reveal that deletion of RecA prevents the effective evolution of nitrofurantoin resistance, and suggest it is a promising therapeutic target, but further refinement of inhibition strategies is needed. This work advances understanding of antibiotic resistance evolution and informs future approaches to constrain bacterial adaptation.

P078

Influence of Growth Media on Collateral Sensitivity and Resistance in E. coli

Prerna Singh, Maria Boubia, Brandon Findlay

Concordia University, Montreal, Quebec, Canada

Abstract

Antibacterial susceptibility testing (AST) is conducted following established guidelines to detect resistance and predict the clinical effectiveness of specific antibiotic treatments. To ensure a controlled environment for bacterial growth and the evaluation of antibacterial efficacy, cation-adjusted Mueller Hinton Broth (MHB) is commonly used as the standard bacteriological medium. However, these guidelines and media formulations do not account for the diverse physiological conditions that can influence the in vivo efficacy of antibacterial agents.

Urinary tract infections (UTIs) are among the most prevalent bacterial infections, posing a significant burden on both healthcare systems and antibiotic usage. In this study, we developed nitrofurantoin-resistance *E. coli* ATCC 25922 mutants *(n=16)* using soft agar gradient evolution (SAGE) separately in two different media—MHB and artificial urine medium (AUM). SAGE creates a continuous antibiotic gradient, allowing bacteria to gradually adapt and develop resistance. AST analysis of these mutants showed a higher minimum inhibitory concentration in AUM than in MHB for three antibiotics: nitrofurantoin, levofloxacin, and trimethoprim. Additionally, the nitrofurantoin-resistant mutants developed in MHB exhibited collateral sensitivity, whereas those evolved in AUM showed cross-resistance.

These findings highlight the importance of considering physiological conditions at the infection site when selecting media for MIC determination to ensure more clinically relevant susceptibility testing.

P079

Phage vs Phage: how prophages contribute to antiviral defence in Pseudomonas aeruginosa

Anna Olina[1], Aleksei Agapov[1], Xinyan Yu[1,2], Stineke van Houte[1], Edze Westra[1]

[1]University of Exeter, Penryn, United Kingdom. [2]Nanjing Medical University, Nanjing, China

Abstract

Phages, the most abundant biological entities on the planet, play key roles in microbial evolution and the composition and function of microbial communities. Recent discoveries highlight that bacteria dedicate a significant portion of their genome to antiphage defences. Anecdotally, defence genes can be located on prophages (phages integrated into the bacterial genome), but systematic studies on their role in bacterial immunity are lacking.

This project aims to unveil the role of prophage-encoded defences in bacterial resistance against lytic phages. Using Pseudomonas aeruginosa as a model, we isolated temperate phages from 500 clinical isolates and generated lysogens in PAO1 lab strain where prophages and defence systems were deleted. We challenged our lysogens with more than 100 diverse temperate and lytic phages to identify defensive phenotypes. Our results indicate that more than half of the prophages confer resistance to other phages. The range of resistance varies across the lysogen collection from only a few to nearly 60% of all phages tested. Sequencing the prophages allowed us to identify resistance patterns according to phylogeny, revealing that closely related prophages often, but not always, provide similar resistance. By integrating experimental data with bioinformatic analyses, we aim to identify key genetic determinants responsible for antiphage resistance.

P080

Phage Tricks and Mimicry Magic: Hunting for Anti-Defense Genes

Samriddhi Gupta, Anna Olina, Aleksei Agapov, Stineke Van Houte, Edze Westra

University of Exeter, Penryn, United Kingdom

Abstract

Phages infect bacteria, which in turn use defense systems to block infection. In response, phages encode anti-defense genes. While over 50 such genes have been identified, many more likely remain undiscovered.

Recent studies show that anti-defense genes often cluster in genomic hotspots. To explore this, we analysed a deduplicated dataset of over 6,000 phage genomes infecting Pseudomonas aeruginosa. Using protein clustering and network analyses, we identified over 80 conserved anti-defense hotspots flanked by consistent bordering genes. We then scanned the full dataset for all regions bounded by these flanking genes (regardless of whether known anti-defense genes were present) and pooled the proteins within them for downstream analyses. Many of these proteins lack functional annotations. Based on domain and protein interaction predictions, several have been prioritised as novel anti-defense candidates.

We also observed orphan defense gene components (individual elements of bacterial defense systems found without their full operons) within these hotspots. Structural predictions suggest that some orphans are truncated versions of their cognate defense genes, lacking catalytic domains but retaining interaction interfaces. We hypothesise that these proteins may act as molecular mimics, binding to host defense partners and sequestering them into non-functional complexes. In some cases, the co-occurrence of orphan genes with anti-defense genes suggests possible functional synergy.

We are currently validating these predictions experimentally and continuing the discovery of new candidates across our dataset. Our findings offer a framework for uncovering novel phage anti-defense strategies and suggest that mimicry of host defense components may be a widespread anti-defense tactic.

P081

Determining the regulation of the newly discovered anti-phage defence system MADS.

Jasmine Thomas-Campbell[1], Anna Olina[2], Aleksei Agapov[2], Stineke Van Houte[2], Edze Westra[2], Tiffany Taylor[1]

[1]University of Bath, Bath, United Kingdom. [2]University of Exeter, Exeter, United Kingdom

Abstract

Bacterial defence systems play a role in the spread of antimicrobial resistance and offer protection against phage infection. Recent work has demonstrated that a newly discovered eight gene defence system MADS cooperates with CRISPR-Cas to protect *Pseudomonas aeruginosa* cells from phage infection. Such a complex system must be tightly regulated by a yet unknown mechanism. Bioinformatic analysis currently suggests that the *mad1* gene from the MADS operon is a transcriptional repressor involved in its regulation.

This study aims to determine whether *mad1* functions as a negative regulator of MADS gene expression and how its activity responds to selective pressures. To investigate the role of *mad1* in regulating MADS, we used qRT-PCR to measure expression levels of MADS genes across a panel of knockout strains derived from a clinical isolate of *Pseudomonas aeruginosa*(SMC4386), including a *mad1* deletion and a defenceless mutant expressing *mad1* from a rhamnose-inducible promoter. We examined gene expression across different induction levels and genetic backgrounds to determine is mad1 is in fact a repressor.

This work will reveal how bacteria regulate the expression of defence systems which is key to predicting the evolution and persistence of phage resistance. Insights into these regulatory mechanisms will advance broader understanding of microbial immune systems and how regulatory networks shape bacterial responses to phage predation. In the age of antimicrobial resistance this work can shed light on predicting and manipulating phage resistance levels in bacterial pathogens.

P082

The cooperation inside us: investigating bacterial cooperation in the human gut

Zohar Katz

University of Oxford, Oxford, United Kingdom

Abstract

The bacteria in our gut are thought to play a major role in our development, behaviour and disease. Rather than acting in isolation, bacteria are extremely social. Cells secrete molecules which benefit nearby cells, with this cooperation allowing the invasion of hosts and acquisition of nutrients. However, the evolution of cooperation is not well understood in our gut communities. Particularly, it is unclear what kind of selection pressures influence cooperation in gut bacterial communities. Using bioinformatics and population genetics, we analysed gut metagenomic data from 231 humans of 98 bacterial species. We calculated the species' genetic relatedness, polymorphism and protein divergence of genes encoding for cooperative and private traits. We found that the number of cooperative genes varied greatly between species, and the proportion of cooperative genes is largely explained by phylogenetics rather than genetic relatedness. We then examined whether genetic diversity in these genes was correlated with the relatedness of species. Theory suggests that genes for cooperation should be under relaxed selection, which means that diversity in these genes (relative to private traits) would increase as relatedness decreases. However, our preliminary results illustrate that species with higher relatedness showed higher relative polymorphism in genes for cooperative compared to private traits. This suggests that other factors at play. For example, selection on cooperation in the gut might be different compared to other systems studied so far. Further investigation is under way to find out. Understanding the social evolution of the gut bacteria might open new avenues to treat microbiome-related conditions.

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P083

Why are antibiotic resistance genes mobile?

Eliza Rayner

University of Oxford, Oxford, United Kingdom

Abstract

Antibiotic resistance genes are frequently carried on and disseminated by conjugative plasmids. Despite their clinical importance, we do not fully understand the evolutionary forces that maintain antibiotic resistance plasmids, or when and how conjugation affects their evolutionary success.

I used experimental evolution to test how two factors influence the importance of conjugation for plasmid stability - the extent of immigration by plasmid-free cells and the strength of positive selection for plasmid-encoded traits. I compared the stability of a conjugative plasmid and a non-conjugative variant in an *E.coli* host, serially passaging cultures over 18 days under different antibiotic concentrations and levels of immigration.

I found support for my hypotheses that conjugation is most beneficial to plasmid stability when immigration is high and positive selection for plasmid carriage is weak. I also compared the fitness of my ancestral and evolved populations using competition assays, providing further insights into the mechanisms underpinning my results. This work contributes to important efforts to better understand why conjugative plasmids play such a key role in the global AMR crisis.

P084

EVALUATION AND ASSESSMENT OF THE BIODEGRADATIVE CAPABILITY OF *Aspergillus flavus* ISOLATED FROM CRUDE OIL CONTAMINATED SOIL IN THE NIGER DELTA REGION OF NIGERIA

Bukola Popoola, Bunmi Oluwatusin, Olusola Olayiwola

Ajayi Crowther University, Oyo, Nigeria

Abstract

Crude oil spillage constitute a major challenge in the Niger Delta Region of Nigeria and this has become a significant threat to farmlands and settlements. Exposure to pollutants can act as a strong selective pressure, favouring the survival and reproduction of microorganisms with advantageous mutations that allow them to tolerate or even utilize the pollutants. This study was carried out to evaluate and thoroughly assess the biodegradative capability of *Aspergillus flavus* from crude oil contaminated soil.

The isolate was molecularly identified and screened for oil degradation in a lab-scale bioremediation study. The ability of the isolate to degrade crude oil was evaluated at five-day intervals for 25 days using mineral salt medium. Polyaromatic hydrocarbons in the crude oil was determined during the degradation period using Gas Chromatography Mass Spectrophotometry (GC-MS). Initial catabolic genes of two petroleum compounds were investigated.

Comparison of the ITS regions of rRNA gene sequence for strain (BMS1) to published sequences in the GeneBank database and phylogenetic analysis, identified BMS1 as *Aspergillus flavus,* which utilized 8 % of crude oil during screening. BMS1 degraded the aromatic compounds in the crude oil from 9.25 ppm to 5.39 ppm by the 25^{th} day. PCR experiments with specific primers for catabolic genes of *alkB* and *nahAc* suggested that BMS1 possess genes for aliphatic (*alkB* gene amplification at about 766bp), while *nahAc* gene was not detected.

Aspergillus flavus, demonstrated high ability to tolerate and utilize crude oil, it could therefore be employed in areas of need.

P085

Decoding Host–Plasmid Interactions: A Genome-Wide Knockout Approach

Jeremy Dejardin, Aleksandra Pokora, Mato Lagator

University of Manchester, Manchester, United Kingdom

Abstract

Plasmids shape microbial evolution by spreading adaptive traits like antimicrobial resistance. However, their impact on bacterial fitness stems from complex, poorly understood interactions between plasmid and host genes.

Here, we examined how host genes influence the fitness effects of plasmid carriage by introducing a large clinical plasmid into ~4,000 *Escherichia coli* single-gene knockouts and tracking growth over 24 hours. We compared fitness impacts in each knockout to wild-type *E. coli* by estimating lag phase, growth rate, and area under the curve (AUC). Functional analyses identified gene groups enriched among knockouts with distinct fitness effects and strains that resisted transformation.

The plasmid increased wild-type fitness, suggesting it carries growth-promoting genes. Unexpectedly, most knockouts reduced this benefit, showing that much of the genome is needed to exploit the plasmid's advantages. Knockouts in membrane and transport genes lowered AUC and growth rate, highlighting nutrient uptake as critical for sustained growth. Deletions in transcriptional regulators and DNA-binding genes extended the lag phase, implying transcriptional flexibility is vital for rapid metabolic shifts. Metabolic genes were enriched among strains that resisted plasmid uptake, suggesting they are required to tolerate the plasmid's burden.

This genome-wide screen identifies key factors for successful plasmid maintenance: metabolic capacity, transcriptional control, and nutrient transport. By pinpointing host gene functions that enable or restrict plasmid persistence, this work deepens understanding of microbial evolution and the spread of antimicrobial resistance through horizontal gene transfer.

P086

Inside the Biofilm: How does *Klebsiella pneumoniae* evolve in catheter-associated urinary tract infections?

Dorothy Cyril-okoh, Jan-Ulrich Kreft, Michelle M.C. Buckner

University of Birmingham, Birmingham, United Kingdom

Abstract

Catheter-associated urinary tract infections (CAUTIs) are frequently (~10-20%) driven by *K. pneumoniae* biofilms, which are known to enhance resistance and persistence. Prior research by Buckner lab detailed biofilm formation and plasmid transfer in *K. pneumoniae* in static single cycles (Element et al., 2023), but CAUTI are in flow conditions and multiple cycles. Therefore, evolutionary dynamics under prolonged catheter-like conditions remain unexplored.

We will simulate indwelling catheter biofilm conditions using an artificial urine flow model, sustaining *K. pneumoniae* growth through ~75 serial passages (~75 days). At predetermined intervals, we will perform whole-genome sequencing, quantify biofilm biomass (crystal violet assay), and measure shifts in resistance. In parallel, we will develop and calibrate a mathematical model built on coupled ODEs with potential fractional-order extensions to potentially capture population dynamics, mutation emergence, biofilm maturation, and antibiotic responses.

We expect to identify parallel emergence of adaptive mutations in genes related to capsule, fimbriae, efflux, and iron uptake aligning with previous research. Evolved biofilms should display increased biomass and altered susceptibility profiles. The mathematical model is expected to recapitulate experimental work, highlight key mutations, and simulate how intermittent antibiotic and selected antiplasmid compounds exposure influences evolution in CAUTI.

This combined experimental and mathematical modeling framework will be the first to integrate evolution of *K. pneumoniae* in CAUTI-relevant biofilms over prolonged exposure. By leveraging experimental and predictive modelling, it lays groundwork for strategies targeting biofilm-adapted, drug-resistant strains in complex environments.

I am currently in the lab running experiments and would present the generated results during the conference.

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