The Abdus Salam International Centre for Theoretical Physics

IAEA International Atomic Energy Agency

unesco United Nations Educational, Scientific and Cultural Organization

Workshop on Plasmids as Vehicles of AMR Spread | (SMR 3876)

18 Sep 2023 - 22 Sep 2023
ICTP, Trieste, Italy

T01 - ARES ARROYO Manuel

Unmasking the Hidden Players: Unveiling the Conjugative Origins of Transfer

T02 - BALTRUS David Anthony

Aeromonas as a major host of antimicrobial resistance in wastewater settings

T03 - BEDHOMME Stephanie

Experimentally exploring the role of codon usage in determining the immediate and evolutionary success of the horizontal transfer of an antibiotic resistance gene.

T04 - BERGIANTE KRAYCHETE Gabriela

Accumulation of replicons carrying multiple copies of mcr and blaKPC-2 in Enterobacter kobei from Brazilian recreational coastal waters

T05 - BUCKNER Michelle Monique

blaNDM-1-bearing plasmid transmission between Klebsiella pneumoniae strains in biofilm and planktonic lifestyle

T06 - CAZARES LOPEZ Adrian

Diverse evolutionary paths led plasmid adaptation to the antibiotic era

T07 - DELGADO BLAS Jose Francisco

The emergence, context and impact of L3 lineage IncL plasmids on antimicrobial resistance dynamics

T08 - DIAZ CABALLERO Julio Felix

Can we use short-read data to monitor AMR markers in plasmids?

T09 - DI MARCO Federico

Exploiting Short-Reads Plasmid Signature for Phenotypic Resistance Analysis: a bioinformatic methodology approach

T10 - DIMITRIU Tatiana

Efficiency of restriction-modification systems against AMR plasmid conjugation

T11 - DORADO-MORALES Pedro Luis

Conjugative-killer plasmids, a novel antimicrobial alternative

T12 - ELSENER Tabea Anna

Three major Neisseria gonorrhoeae beta-lactamase plasmid variants are associated with TEM alleles and lineages

T13 - FAIZRAHNEMOON Mahsa

PlasBin-flow: A flow-based MILP algorithm for plasmid contigs binning

T14 - FROLOVA Daria

Approximating plasmid relatedness using Double Cut and Join Indel model

T15 - GIMÉNEZ MARTINEZ Matías

Improved detection and classification of plasmids from circularized and fragmented assemblies.

T16 - HASMAN Henrik

Plasmid spread from carbapenemase producing Enterobacter hormaechei ST79 present on contaminated Dicillin capsules sold in Denmark

T17 - LAM Margaret

Genomic dissection of plasmids driving AMR and hypervirulence convergence in Klebsiella pneumoniae

T18 - LEON-SAMPEDRO Ricardo

The invasion success by antibiotic-resistant plasmid-bacterium associations in human-associated communities is determined by the interplay between intrinsic and environmental factors

T19 - MATLOCK William Thomas

Integrons on plasmids: the global genomic epidemiology of blaGES-5

T20 - MEDINA MENDEZ Juan Manuel

Transference of antibiotic resistance genes through marine conjugative plasmids

T21 - MOLARI Marco

Capturing microbial genome diversity with pangenome graphs: the case of E. coli ST131

T22 - NONNENMACHER Toby Sean

Investigation of the extent of clonal and multiple acquisition in OXA-48 plasmids in UK hospitals

T23 - PARTRIDGE Sally Rachel

Detecting AMR plasmid variants in Shigella sonnei

T24 - PEÑIL CELIS Arancha

Jaccard Index Network Analysis reveals the contribution of accessory genome to the recent diversification of Salmonella Typhi

T25 - PÉREZ SÁNCHEZ Marco Arturo

IncC plasmids in the emergent genotype ST213 of Salmonella Typhimurium: Phenotypic analyses of plasmid-cured mutant strains and comparative genomics.

T26 - RAABE Nathan Jonathan

Genomic Epidemiologic Investigation of a Hospital Outbreak of NDM-5-Producing Gram-Negative Enterobacterales Infections

T27 - ROBERTS Leah Wendy

RoundHound: detecting plasmid transmission from short-read datasets

T28 - WELKER SAPOJKIN ROSSINE Fernando

The role of intracellular competition in the fixation of novel plasmids.

T29 - SAMUEL Bruria

Anti-Defense Systems on Plasmids

T30 - STALDER Thibault Pierre Gabriel

Identifying plasmid transconjugants in the rare biosphere of the barley rhizosphere using Hi-C and target enrichment

T31 - WAN Yu

Integrated analysis of patient networks and plasmid genomes reveals a regional, multi-species outbreak of carbapenemase-producing Enterobacterales carrying both blaIMP and mcr-9 genes

T32 - WITZANY Christopher Bernhard

Evolutionary interplay of chromosomal and plasmid-borne compensatory mutations

T01

Unmasking the Hidden Players: Unveiling the Conjugative Origins of Transfer

M. Ares-Arroyo [1], and E. P. C. Rocha [1]

[1] *Institut Pasteur, Université de Paris Cité, CNRS UMR3525, Microbial Evolutionary Genomics, Paris, France*

Plasmids are major drivers of bacterial evolution, facilitating the accumulation and spread of antimicrobial resistance (AMR) genes between bacteria. However, despite decades of research, we have little clue on the mobility mechanisms of most plasmids, since they lack the genes required for conjugation ("pMOBless" hereinafter). How these plasmids move has remained a longstanding enigma in the field until pioneering works by Ramsay and collaborators suggested that many pMOBless in *Staphylococcus aureus* could carry conjugative origins of transfer (*oriT*), thus, could potentially be mobilized [1]. Following this work, we analysed the presence of experimentally validated *oriTs* in >2,000 complete genomes of *S. aureus* and *E. coli*, the two leading nosocomial species in terms of AMR-associated deaths. Importantly, *oriTs* were present in most pMOBless of these two species, suggesting that more than >85% of plasmids can transfer via self-conjugation or trans-mobilization [2]. Accordingly, *oriT*-carrying pMOBless were generally present in bacteria bearing multiple-plasmids, presumably due to their functional dependencies of either a conjugative plasmid (like parasites of conjugation) or the interplay between conjugative and mobilizable ones (hyperparasites). Now, we expanded our analysis to all the species represented in RefSeq (>21,000 complete genomes) to ascertain the universality of our previous findings. Conversely, our results revealed the little knowledge regarding *oriTs* outside few well-studied species, as we could only identify an *oriT* in ~40% of conjugative/mobilizable plasmids. To solve this scarce of knowledge, we proceeded with an integrative analysis of the known origins of transfer to infer novel *oriTs* among all bacteria. We investigated their replicon tropism, taxa specificity, genetic location, proximity to the relaxase, relative location to other conjugative genes, and size of the *oriT*-carrying intergenic sequences. Our in-depth analysis led us to the characterization of numerous putative novel *oriTs* outside the well-studied model species, many of these representing the first *oriT* characterizations in their respective species. Our work suggests that most plasmids in bacteria can be assigned with a putative mechanism of mobility. In conclusion, our work helps solving a longstanding enigma in the field, and provides novel insights into the spread of plasmid-mediated AMR between bacteria.

[1] J. P. Ramsay, S. M. Kwong, et al. Mob. Genet. Elements. **6**, e1208317 (2016).
[2] M. Ares-Arroyo, C. Coluzzi, E.P.C. Rocha, Nucleic Acids Res. **51**, 3001-3016 (2023).

T02

We sampled plasmid-host relationships and antimicrobial resistance genes in two paired communities on the border of the United States and Mexico (Yuma, Arizona and San Luis, Mexico) using both Nanopore sequencing and studies with proximity ligation. We found that Aeromonas species are major carriers of antibiotic resistance, with single plasmids containing multiple antimicrobial resistance pathways, within these wastewater samples and could represent in important source for dissemination of resistance to other gram negative species.

T03

Title: Experimentally exploring the role of codon usage in determining the immediate and evolutionary success of the horizontal transfer of an antibiotic resistance gene.

Abstract: Horizontal gene transfer (HGT) is both a major player in bacteria genome evolution and an important mechanism of antibiotic resistance propagation. However genomic data acquired in the last decade reveal that genes do not circulate freely between bacterial genomes. Understanding the horizontal traffic rules of antibiotic resistance genes is key to designing measures to limit their propagation.

Through a multi-species experimental approach, we investigated the potential role of codon usage preferences in determining the success and the evolutionary outcome of horizontal transfers. By inserting plasmids carrying more than 30 synonymous versions of a gentamicin resistance gene in three bacteria species (*Escherichia coli*, *Pseudomonas aeruginosa* and *Acinetobacter baylyi*), we show that synonymous variation has a strong and species-specific impact on the level of resistance provided, supporting the idea that codon usage preferences could be a barrier to HGT under certain circumstances. However, the general codon usage match between the synonymous variants and the receiving genome is not a strong determinant of the level of resistance conferred.

We further conducted experimental evolution of a subset of the synonymous variant * species combinations in presence and absence of gentamicin. In presence of gentamicin, similar levels of resistance evolve for all variants, very likely through compensatory mutations, thus rapidly erasing the differences between variants. In absence of gentamicin, resistance is lost or conserved, in a variant-specific manner and the genetic mechanisms behind this phenomenon are still under investigation.

T04

Accumulation of replicons carrying multiple copies of *mcr* and *bla*$_{KPC-2}$ in *Enterobacter kobei* from Brazilian recreational coastal waters

Gabriela B. Kraychete[1], Larissa A. B. Botelho[1], Raquel R. Bonelli[1], and Renata C. Picão[1]

[1]Instituto de Microbiologia Paulo de Góes, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil.

Polymyxins are the last line of defence in treating infections caused by carbapenem-resistant *Enterobacteriaceae*. Consequently, the global spread of horizontally transferred *mcr* genes, which confer resistance to colistin, is a significant concern for public health worldwide. To our knowledge, reports of the coexistence of *mcr* and carbapenemase-encoding genes in Brazil have been limited to clinical settings. This study focuses on characterizing two *Enterobacter kobei* isolates (BT221 and BT722) obtained from recreational coastal waters in Brazil. These isolates are noteworthy for carrying both *mcr* and *bla*$_{KPC-2}$ antimicrobial resistance genes. Initially, BT221 and BT722 were identified as *Enterobacter cloacae* complex through MALDI-TOF and were found to produce carbapenemase by Carba-NP testing. Here, we evaluated the susceptibility of the strains to various antibiotics using the disc diffusion method, including amoxicillin/clavulanate, ampicillin, aztreonam, cefoxitin, cefotaxime, ceftazidime, cefepime, ertapenem, meropenem, imipenem, nalidixic acid, ciprofloxacin, levofloxacin, chloramphenicol, amikacin, gentamicin, fosfomycin, sulfamethoxazole/trimethoprim, and tetracycline. Colistin susceptibility was assessed through microdilution. Additionally, the strains were submitted to whole-genome sequencing (WGS) using Illumina and Nanopore technologies. Both isolates were identified as multidrug-resistant, exhibiting resistance to β-lactams, fluoroquinolones, and colistin. Notably, they displayed high minimum inhibitory concentrations (MICs) for colistin (32 and >512 μg/mL, respectively). WGS confirmed that both strains were *E. kobei*. Analysis of the resistome revealed acquired resistance determinants against β-lactams, aminoglycosides, colistin, macrolides, quinolones, and trimethoprim in BT221; and additionally against fosfomycin in BT722. BT221 solely carried the *mcr-9* variant, whereas BT722 harbored *mcr-9*, *mcr-5*, and a truncated form of *mcr-10* (with 91.30% coverage). In terms of β-lactamase-encoding genes, both isolates carried *bla*$_{KPC-2}$ and the intrinsic *bla*$_{ACT-9}$, while BT722 also harbored *bla*$_{TEM-1A}$, *bla*$_{OXA-9}$, and *bla*$_{GES-16}$. Plasmid markers IncFII(Yp), IncU, Col440I, and Col440II were identified in BT221, while BT722 exhibited IncU, IncP6, IncQ1, and ColE10 groups. Both isolates carried *mcr-9* on the chromosome. BT221 carried the *bla*$_{KPC-2}$ gene on an IncU plasmid spanning 45,575 bp, which also contained macrolide resistance genes (*msr*(E) and *mph*(E)) and a quinolone resistance gene (*qnrVC1*). Additionally, BT722 harbored *mcr-5*, carbapenemase-encoding genes *bla*$_{KPC-2}$ and *bla*$_{GES-16}$, as well as macrolide (*mph*(E) and *msr*(E)), quinolone (*qnrVC1*), and trimethoprim (*df21*) resistance genes on a hybrid IncU/IncP6 plasmid, which was 41,147 bp long. Furthermore, BT722 also carried the truncated *mcr-10* gene, along with aminoglycoside resistance genes (*aac(6')-Ib* and *ant(3'')-Ia*) and β-lactamase resistance genes (*bla*$_{TEM-1A}$ and *bla*$_{OXA-9}$), on a non-typeable plasmid spanning 157,242 bp. A second copy of the *bla*$_{KPC-2}$ gene was detected on a small IncQ1 plasmid (8,042 bp) in BT722, with high coverage to other *bla*$_{KPC-2}$-carrying IncQ-plasmids found in Brazilian clinical and environmental isolates. The *E. cloacae* complex species are part of the human and animal microbiota and commonly found in environmental sources. Thereafter, the identification of strains belonging to this species carrying various antimicrobial resistance determinants and plasmids in the aquatic matrices, highlights the necessity of understanding the molecular mechanisms contributing to the acquire and maintenance of these genes in environmental strains. Moreover, the discovery of multiple copies of *mcr* and carbapenemase-encoding genes within a single strain from recreational coastal waters raises concerns about the potential dissemination of clinically relevant antimicrobial resistance determinants within the community.

T05

bla$_{NDM-1}$-bearing plasmid transmission between *Klebsiella pneumoniae* strains in biofilm and planktonic lifestyle

Sarah J. Element[1], Robert A. Moran[1], Emilie Beattie[1], Rebecca J. Hall[1], Willem van Schaik[1], Michelle M.C. Buckner[1]

[1]Institute of Microbiology and Infection, College of Medical and Dental Sciences, University of Birmingham, UK.

Antimicrobial resistance (AMR) is a growing problem, especially in Gram-negative Enterobacteriales such as *Klebsiella pneumoniae.* Conjugative plasmids contribute to AMR gene dissemination within and between bacterial species. Bacteria such as *K. pneumoniae* commonly exist in biofilms, yet most studies focus on planktonic cultures. We are studying the transfer of a multidrug resistance plasmid in planktonic and biofilm populations of *K. pneumoniae.* In particular, we study plasmid transfer using a clinical isolate, which was isolated from a patient in the Queen Elizabeth Hospital, Birmingham. This strain carried four plasmids, including a 119-kbp *bla*$_{NDM-1}$-bearing F-type plasmid which we termed pCPE16_3. We found that transfer frequency of pCPE16_3 in a biofilm was orders-of-magnitude higher than between planktonic cells. We have also found that in the majority of our transconjugants multiple plasmids are transferred. Plasmid acquisition had no detectable growth impact on transconjugants. We used RNA-sequencing to examine gene expression of the plasmid-free recipient and a transconjugant in three lifestyles: planktonic exponential growth, planktonic stationary phase, and biofilm. Unsurprisingly, lifestyle had a substantial impact on chromosomal gene expression. In line with our previous work, plasmid carriage affected chromosomal gene expression; this was most striking in stationary planktonic and biofilm lifestyles. Furthermore, expression of plasmid genes was lifestyle-dependent, with distinct signatures across the three conditions. Our study shows that growth in biofilm greatly increased the risk of conjugative transfer of a carbapenem resistance plasmid in *K. pneumoniae* without detected fitness costs and minimal transcriptional rearrangements, thus highlighting the importance of biofilms in the spread of AMR in this opportunistic pathogen.

T06

Diverse evolutionary paths led plasmid adaptation to the antibiotic era

Adrian Cazares [1,2] **, Nick R. Thomson** [1]**, and Zamin Iqbal** [2]

[1] *Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton, Cambridgeshire, CB10 1SA, United Kingdom*

[2] *EMBL's European Bioinformatics Institute (EMBL-EBI), Wellcome Genome Campus, Hinxton, Cambridgeshire, CB10 1SD, United Kingdom*

Plasmids are known vectors of antimicrobial resistance (AMR) genes conferring multidrug resistance (MDR) and thus play a pivotal role in the current AMR crisis. Despite their relevance, little is known about how resistance plasmids originated and, more broadly, how antibiotics influenced plasmid evolution in the first place. By taking modern plasmids and unique specimens from the pre-antibiotic era (PAE), isolated worldwide between 1917 and 1954, we explored the incidence and diversity of plasmids in pathogens before the introduction of antibiotics for therapy, and whether antibiotics induced a shift in plasmid diversity and/or gene content in modern pathogens. We uncovered more than 700 novel plasmid sequences and show that these elements were diverse and prevalent in PAE pathogens. Our comparative analysis revealed that these PAE plasmids are related to ~10000 modern plasmids residing in numerous pathogenic species isolated across six continents, thus indicating that PAE plasmids are still in circulation today and have a global distribution. By integrating network and functional analyses, we elucidated the population structure of PAE-modern plasmids, discovering hundreds of lineages and their relative contribution to AMR dissemination. No AMR genes were detected in PAE plasmids; however, we identified various lineages featuring MDR plasmids. We also uncovered the concomitant acquisition of other genes involved in gene mobility and virulence in modern plasmids. Remarkably, we show that not all lineages have evolved AMR carriage. Our study provides an unparalleled view of plasmid adaptation to the antibiotic era and reveals genetic features linked to the origin of resistance plasmids.

T07

The emergence, context and impact of L3 lineage IncL plasmids on antimicrobial resistance dynamics

Jose F. Delgado-Blas [1], Sophia David [2], Carlos Serna [1], Mario Pulido-Vadillo [1], Natalia Montero [1], David M. Aanensen [2], Lorena Abadia-Patiño [3], Bruno Gonzalez-Zorn [1]

[1] Antimicrobial Resistance Unit (ARU), Faculty of Veterinary Medicine and VISAVET Health Surveillance Centre, Complutense University of Madrid, Madrid, Spain
[2] Centre for Genomic Pathogen Surveillance (CGPS), Big Data Institute, Li Ka Shing Centre for Health Information and Discovery, University of Oxford, Oxford, UK
[3] Bacterial Resistance Laboratory, Department of Biomedicine, Institute for Research in Biomedicine and Applied Sciences, University of Oriente (IIBCAUDO), Cumaná, Venezuela

IncL plasmids constitute one of the most clinically relevant plasmid subtypes due to their role in antimicrobial resistance (AMR) dynamics. Five IncL lineages have been described to date (L1-L5) [1]. L4 is the most frequent lineage, formed by the prominent pOXA-48 plasmids, followed by the unknown L3 lineage. Thus, the aim of this work was to determine the genomic and epidemiological features of IncL lineages and their association with AMR genes, with a special focus on the L3 lineage. A conjugative L3 plasmid responsible for the dissemination of the novel *rmtE4* gene, conferring resistance to most clinically used aminoglycosides, was identified in *Klebsiella* spp. isolates from South America [2]. An IncL plasmid collection (n=169) was created with close NCBI sequences. IncL plasmids were classified into specific lineages with a L-type replicon database [1]. The collection was screened for AMR genes using ResFinder database. Pan-genome analyses were performed with the Roary pipeline, extracting the SNPs from core-genes and generating a core-genome SNP-tree with RAxML. Plasmids belonging to L3 lineage were aligned in a whole-length comparison to evaluate their genetic content and organization. Pan-genome analysis revealed the large and diverse genetic content of IncL plasmids (36 core-genes/494 gene clusters, 7.3% core-genome). This genetic diversity was mainly due to L4 lineage (38 core-genes/430 total genes, 8.8% core-genome), whereas L3 lineage core-genome was much larger (78/133, 58.65%). However, this contrast could be caused by the enormous overrepresentation of pOXA-48 plasmids. AMR genes were among those conferring this genetic variability in L4 plasmids (26 genes), but most of them only carried $bla_{\text{OXA-48}}$ (79.3%). However, L3 lineage showed a lower AMR gene diversity (15 genes), due to its limited representation, but all of them carried three or more AMR genes. In contrast with L4 lineage, L3 plasmids were highly conserved, with only three core-genome SNPs, differentiating two subclusters. One subcluster from *Enterobacteriaceae* isolates collected in a single hospital in the USA [3] and the South American subcluster harbouring *rmtE4*. Regarding the L3 accessory-genome, some mobile genetic elements and AMR genes were shared by all members, such as the $bla_{\text{TEM-1B}}$-IS*26* module. However, some genetic modules were subcluster-specific, including the $bla_{\text{KPC-2}}$-IS*Kpn* elements in USA hospital isolates and the *rmtE4*-IS*Vsa3* in South American plasmids. The phylogenetic link, the genetic content and the geographical distribution of these subclusters suggested a common plasmid ancestor followed by a parallel diversification. The integration and fixation of *rmtE4* into L3 plasmids associated with hospital settings entails a significant risk for the dissemination of this gene to other regions worldwide.

[1] G.A. Blackwell, E.L. Doughty, R.A. Moran, Plasmid. **113**, 102528 (2021).
[2] J.F. Delgado-Blas, C.M. Ovejero, Microb. Genom. **9(3)**, mgen000946 (2023).
[3] R.A. Weingarten, R.C. Johnson, mBio. **9(1)**, e02011-17 (2018).

T08

Can we use short-read data to monitor AMR markers in plasmids?

Julio F. Diaz Caballero [1,2] , **Natacha Couto** [1] , **Sophia David** [1] , **and David Aanensen** [1,2]

[1] *Centre for Genomic Pathogen Surveillance, Big Data Institute, Nuffield Department of Medicine, University of Oxford, UK*

[2] *WHO Collaborating Centre on Genomic Surveillance of Antimicrobial Resistance, University of Oxford, UK*

Long-read and hybrid assemblies of microbial genomes are seen as the standards to determine plasmidic carriage of AMR genes because these assemblies can distinguish chromosomes and plasmids more effectively, but also because AMR markers are often found in regions of the genome that are difficult to contiguously assemble with short reads, *e.g.* transposons. Nevertheless, the availability of long-read sequencing is limited, and public data is largely generated with short-read sequencing technologies. Currently, less than 3% of the data in the public archives come from long-read sequencing technologies. Moreover, wide adoption of long-read sequencing is slow, and genomic surveillance of AMR may have to wait beyond the short-term to benefit from it. Short-read data is more widely available, and it benefits from longer historical archives. Thus, we aim to measure and identify the limitations of AMR plasmid surveillance using short-read sequencing data.

Modern bioinformatics tools take us a step closer to accurately identifying plasmids using short-read sequences, albeit their unalienable limitations, but `how close?` is still an unanswered question. Here, we use a dataset of bacterial genomes, which have been sequenced with long- and short-read sequencing, and compare their short-read assemblies to their hybrid equivalent from the perspective of plasmid identification. Specifically, we measured the accuracy of three algorithmically distinct tools, namely Platon [1], MOB-suite [2], and plasmidEC [3], at identifying short-read contigs as plasmidic. In addition, we determined the effect of short-read assembly discontinuity on the identification of AMR markers in plasmids. It is possible that these tools may well correctly identify plasmid sequences, but that the AMR content of plasmids is missed due to assembly limitations or library preparation challenges. Potential use of short-read data in the monitoring of plasmids as vehicles for AMR would need to take into account these limitations.

Our preliminary results show that 1) hybrid assemblies don't always yield distinct complete replicons, 2) these tools are more accurate at identifying chromosomal sequences than plasmidic ones, and 3) higher accuracy is observed when combining results from these tools.

[1] O. Schwengers, P. Barth, L. Falgenhauer, T. Hain, T. Chakraborty, A. Goesmann, Microb. Genom. **6(10)**, mgen000398 (2020).

[2] J. Robertson, J.H.E. Nash, Microb. Genom. **4(8)**, e000206 (2018).

[3] L. Vader, J. Paganini, J. Kerkvliet, A. Schurch, Unpublished. **https://gitlab.com/mmb-umcu/plasmidEC** .

T09

Exploiting Short-Reads Plasmid Signature for Phenotypic Resistance Analysis: a bioinformatic methodology approach

F. Di Marco[1], F. Saluzzo[1,2], G. Lorenzin[1], A. Spitaleri[1,2], M. Rossi[3], F. Gona[1], V. Batignani[1], R. Sorrentino[1], A. Gori[4], D. M. Cirillo[1], KPC-Kp Study Group

[1]IRCCS Ospedale San Raffaele, Emerging Bacterial Pathogen Unit, Milano
[2]Università Vita Salute San Raffaele, Milano
[3]Ospedale San Gerardo, UO Malattie Infettive, Monza
[4]Università degli Studi di Milano, Dipartimento di Fisiopatologia Medico-Chirurgica e dei Trapianti, Milano

Understanding the role of mobile genetic elements (MGEs) in the transmission of antimicrobial resistance (AMR) is crucial for effective surveillance and prevention efforts. However, current whole genome sequencing (WGS) methods have limitations in accurately predicting AMR. In this study, we propose an innovative approach that utilizes short-read sequencing platforms to establish a unique "plasmid signature" for *Klebsiella pneumoniae* (KP) isolates.
We analysed the genomic content of over 700 KP-KPC isolates obtained from 15 Lombardy hospitals and compared them with a reference database of 4000 plasmid sequences from PLSDB repository [1].
Isolates' assembled contigs and reads were matched with the reference dataset discovering a concurrence with 253 reference plasmid sequences. The combination of covered plasmid fractions allowed us to assign a distinct "plasmid signature" to each isolate. Then, an unsupervised K-means algorithm was employed to classify the strain using the plasmid signature as the main parameter. The k-means algorithm defined four "plasmid signature" based groups (PSGs).
The phenotypic characterizing revealed that each PSG has a unique resistance profile.
Group 1, primarily consisting of ST307 strains, exhibits susceptibility to amikacin (AK) but has a high level of resistance to sulfamethoxazole/trimethoprim (S/T). Group 2, consisting of ST101 and ST307 strains, is moderately susceptible to all drugs (AK, S/T, and gentamicin).
Group 3 is the largest and it's mainly composed of ST512 and ST258; this group exhibits a high level of resistance to AK. Group 4 is characterized by a high level of resistance to both AK and S/T and most of the strains linked in this group are reported as ST512.
To validate our plasmid signature approach, we employed a long-read sequencing-based approach using Oxford Nanopore Technologies (ONT). Ten isolates, representatives of all groups, were sequenced with ONT; then, the classifications obtained with both sequencing techniques were compared. The comparison confirmed the reliability of our approach. Furthermore, the analysis revealed unique genetic content within plasmids carrying the same replicons, including various versions of the blaKPC gene.
This methodology emphasizes the potential of plasmid signatures in AMR classification. By focusing on plasmid content, we were able to differentiate KP groups with specific resistance profiles. These findings suggest that plasmid signature analysis can enhance AMR surveillance and provide valuable insights for epidemiological retrospective investigations in settings where ONT is not available.

[1] G.P. Schmartz, A. Hartung, P. Hirsch, F. Kern, T. Fehlmann, R. Müller, A. Keller. Nucleic Acids Res, **50**, D273–D278, doi:10.1093/NAR/GKAB1111 (2022)

T10

Efficiency of Restriction-Modification systems against AMR plasmids

T. Dimitriu[1] **, M.D. Szczelkun**[2] **, and E.R. Westra**[1]

[1]*ESI, Biosciences, University of Exeter, TR10 9FE Penryn, UK*
[2]*School of Biochemistry, University of Bristol, BS8 1TD Bristol, UK*

In bacteria, genes conferring antibiotic resistance are mostly carried on plasmids, mobile elements which spread horizontally among bacterial hosts. Bacteria carry immune systems to defend against genetic parasites, but how they act against plasmids, and in particular how effective they are against conjugative transmission, is poorly understood.
Here, we measured how effective bacterial restriction-modification (RM) immune systems are against a collection of natural, conjugative antibiotic resistance plasmids. We uncovered variation in defense efficiency ranging from none to 10^5-fold protection, depending on both RM system and plasmid type. We then explored the contribution of RM type, plasmid escape, and plasmid anti-RM genes to this variation. As expected, we find that restriction efficiency depends on the number of recognition sites present on the conjugative plasmids. However, this pattern does not hold for type I restriction systems. In addition, most plasmids carry one or several genes encoding putative anti-restriction proteins, which are known to target type I restriction systems. We are currently testing experimentally the effect of these genes on restriction.

T11

Conjugative-killer plasmids, a novel antimicrobial alternative

Pedro Dorado-Morales [1*], **Morgan Lamberioux** [1*], **Jakub Czarnecki** [1] **and Didier Mazel** [1]

[*] These authors contributed equally to this work.

[1] *Unité Plasticité du Génome Bactérien, CNRS UMR 3525, Institut Pasteur, 28 rue du Dr Roux, 75015 Paris, France*

Targeted killing of pathogenic bacteria without harming beneficial members of host microbiota holds promise as a strategy to cure disease and limit both antimicrobial-related dysbiosis and development of antimicrobial resistance. Recent work from our lab has demonstrated that genetic modules based on toxin-intein systems delivered by conjugation are highly effective antimicrobials agents, able to selectively kill *Vibrio cholerae* in mixed populations [1]. In this line of work, we are adapting the aforementioned system to other pathogens of clinical importance by including new toxin modules whose expression depends on transcriptional factors that are exclusively present in the targeted bacteria. The three chosen bacteria and their selected specific transcriptional regulators are: *Salmonella enterica* and HilD, the master regulator of the invasion process [2]; *Klebsiella pneumoniae* and YbtA, the central regulator of yersiniabactin production, the most common high-virulence determinant [3]; *Shigella flexneri* and enteroinvasive *Escherichia coli* and VirF, the primary regulator of the virulence phenotype in both species [4]. Additionally, to ensure an efficient dissemination and maintenance across the microbial gut population, we are engineering conjugative plasmids, such as RP4, to be transferred and maintained between enterobacteria and *Bacteroides*, one of the main constituents of the gut microbiome. Once validated under laboratory conditions, the system will be assayed in mock complex populations. The results obtained from these preliminary tests will direct the refinements needed for the tool to be effective in a real scenario.

[1] López-Igual, R., *et al*. (2019). Nature Biotechology 37(7):755-760
[2] Smith, C., *et al*. (2016). mBio 7(5)
[3] Lam, M., *et al*. (2018). Microbial Genomics 4(9)
[4] Lan, R., *et al*. (2003) Infection and Immunity 71(11):6298-6306

T12

Three major *Neisseria gonorrhoeae* β-lactamase plasmid variants are associated with TEM alleles and lineages

Tabea A. Elsener[1], Keith A. Jolley[2], Eduard A. Sanders[3], Martin C. J. Maiden[2], Ana Cehovin[1] and Christoph M. Tang[1]

[1] Sir William Dunn School of Pathology, University of Oxford, UK
[2] Department of Biology, University of Oxford, UK
[3] Arum Institute, Johannesburg, South Africa and KEMRI-Wellcome Trust Research Programme, Kilfi, Kenya

Without a vaccine, the management of gonorrhoea depends on effective antibiotic treatment. However, *Neisseria gonorrhoeae* (the gonococcus) has acquired resistance to most available antibiotics [1], leaving cephalosporins as the last resort treatment [2, 3]. The gonococcal β-lactamase plasmid p*bla* encodes the TEM β-lactamase, which requires only one to two amino acid changes to become an extended-spectrum β-lactamase, conferring resistance against cephalosporins [4]. p*bla* is mobilisable by the gonococcal conjugative plasmid pConj [5], which leads to the spread of β-lactamase-mediated resistance.

Several variants of p*bla* have been reported [6-8], which can differ in the TEM allele they carry and their ability to be spread by pConj. However, large-scale analyses of the distribution of plasmid variants are lacking due to repeat sequences on the plasmid, hindering its assembly from short-read sequences. We have devised the p*bla* typing scheme Ng_p*bla*ST and implemented it on the PubMLST database [9] to assess the spread of variants across 15 532 isolates. This revealed three major variants associated with distinct TEM alleles, gonococcal lineages and pConj variants.

To gain insights into the molecular determinants of the spread of p*bla* variants, we investigated the impact of the variant-specific deletions on plasmid transfer and the interplay between p*bla* and pConj variants. We identified distinct mobilisation patterns of p*bla* variants and differences in the ability of pConj variants to facilitate this transfer. Linking the variation and distribution of p*bla* variants to their transfer ability and identifying selection pressures favouring distinct variants is important to monitor and predict the spread of β-lactamase-resistance resistance in *N. gonorrhoeae*.

[1] Unemo M, Golparian D, Eyre DW. Methods Mol Biol. **1997**:37-58. (2019)
[2] Hazra A, Collison MW, Davis AM. JAMA. **327**:870–871. (2022)
[3] Fifer H, Saunders J, Soni S, Sadiq ST, FitzGerald M. Int J STD AIDS. **31**:4–15. (2020)
[4] Salverda MLM, De Visser J, Barlow M. FEMS Microbiol Rev. **34**:1015–1036. (2010)
[5] Baron ES, Saz AK, Kopecko DJ, Wohlhieter JA. Antimicrob Agents Chemother. **12**:270–280. (1977)
[6] Pagotto F, Aman AT, *et al*. Plasmid. 43:24–34. (2000)
[7] Müller EE, Fayemiwo SA, Lewis DA. J Antimicrob Chemother. **66**:1514–1517. (2011)
[8] Trembizki E, Buckley C, et al. Antimicrob Agents Chemother. **58**:4984–4985. (2014)
[9] Jolley KA, Bray JE, Maiden MCJ. Wellcome Open Res. **24**;3:124. (2018)

T13

PlasBin-flow: A flow-based MIL

plasmid contigs binni

Aniket Mane , Mahsa Faizrahnemoon, Tomáš Vinar , Brona Bre

Department of Mathematics, Simon Fraser University, Canada
Department of Applied Informatics, Comenius University, Slovakia
Department of Computer Science, Comenius University, Slovakia.

GenomeCanada

NSERC CRSNG

SFU

Plasbin-flow

- Problem: grouping contigs into bins, representing groups of contigs likely originating from the same plasmid.

- Method:
- **Assembly graph:** vertices of this graph are called contigs and represent assembled sequences, and edges encode potential contiguity of the contigs along the genome of origin.
- **Optimization based approach**: using Mixed Integer Linear Programming (MILP) techniques.
- Maximizes the objective function defined based on plasmid features namely **read depth**, plasmid **gene density** and **GC content similarity**.
- Using **network flows**.

A

Evaluation metrics and

Precision: Each predicted bin is matched to the most similar true plasmid

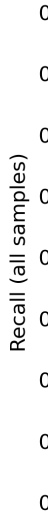
Recall: Each true plasmid is matched to the most similar predicted bin

F1: Harmonic mean of the precision and recall

Tool	Precision	Recall	F1
Plasbin-flow	**0.98**	0.89	**0.93**
Plasbin	0.72	0.58	0.58
HyAsp	0.96	0.84	0.88
plasmidSpades	0.48	0.63	0.50
Mob-recon	0.92	**0.97**	0.91

PlasMerge: Post proce

- Given a set of plasmid bins, decide which sets of bins to merge into a single bin
- Combinatorial optimization method to connect them.
- Consider all pairs of bins to merge and weight the path between them by the decrease in the objective function



- Rene Roy: *Department of Mathematics, University of Waterloo, Canada*

Thank you ☺

T14

Approximating plasmid relatedness using Double Cut and Join Indel model

Daria Frolova[1] , **Leandro Lima**[1] , **Leah Roberts**[2] , **and Zamin Iqbal**[1]

[1]*European Molecular Biology Laboratory, European Bioinformatics Institute, Hinxton, United Kingdom*
[2] *Centre for Immunology and Infection Control, Queensland University of Technology, Brisbane, Queensland, Australia*

The comparatively rapid structural changes in plasmid genomes present considerable challenges to evolutionary and epidemiological analysis. Sophisticated maximum likelihood and Bayesian approaches, as used in phylogenetics, are not available as we do not have good evolutionary models.
Given that, the typical approach is to base analysis on shared sequence content, either via aligned blocks or shared kmers, and use this to define a genetic distance. These approaches create artefacts with plasmids of different size, which is a significant limitation given gene gain/loss is a common process, and plasmids sometimes fuse/cointegrate. Furthermore, the numeric values are not interpretable in terms of genetic events separating a pair of plasmids.
We therefore introduce a model which captures the key components of how plasmid genomes evolve structurally – through gene/block gain or loss, and through rearrangement. We use the "Double Cut and Join Indel" model for measuring rearrangement distances between genomes, which Bohnenkamper et al recently solved [1]. Plasmids are studied at a coarse level, as a sequence of integers (representing genes or aligned blocks), and the distance between two plasmids is the minimum number of rearrangement events (insertion, deletion, or "double cut and join") between them. We show how this gives much more meaningful distances between plasmids, coping with related plasmids that would seem very unrelated by other methods.
We apply the method to the recent study of Matlock et al [2], showing how structural and SNP distances give complementary information on a clade of plasmids seen in multiple niches (human and animal hosts.)

[1] L. Bohnenkämper, M. D.V. Braga, D. Doerr, and J. Stoye, Journal of Computational Biology **28:4**, 410-431 (2021)
[2] W. Matlock, S. Lipworth, K. K. Chau, M. AbuOun, L. Barker, J. Kavanagh, M. Andersson, S. Oakley, M. Morgan, D. W. Crook, D. S. Read, M. Anjum, L. P. Shaw, N. Stoesser, REHAB Consortium, UK eLife **12**, e85302 (2023)

T15

Improved detection and classification of plasmids from circularized and fragmented assemblies.

Matías Giménez [1,2,3], **Ignacio Ferrés** [1], **and Gregorio Iraola** [1,3,4,5]

[1] *Microbial Genomics Laboratory, Institut Pasteur Montevideo, Uruguay.*

[2] *laboratorio de Microbiología Molecular , Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay.*

[3] *Center for Innovation in Epidemiological Surveillance (CiVE), Institut Pasteur Montevideo, Uruguay.*

[4] *Wellcome Sanger Institute, Hinxton, Cambridge, CB10 1SA, United Kingdom.*

[5] *Center for Integrative Biology, Universidad Mayor, Santiago de Chile, Chile*

Plasmids are mobile genetic elements important for bacterial adaptation. The study of plasmids from sequencing data is challenging because short reads produce fragmented assemblies, requiring of subsequent discrimination between chromosome and plasmid sequences. Although circularized assemblies are now possible using long-read data, there is still a lot of information to be retrieved from short-read metagenomic contigs. Here, we present plaSquid, a dockerized tool developed in Nextflow that expands plasmid detection and improves replicon typing classification schemes, outperforming previously available methods in both precision and sensitivity. When applied to ∼10.5 million metagenomic contigs, plaSquid revealed a 2.7-fold increase in plasmid phylogenetic diversity. Also, we used plaSquid to uncover a significant role of plasmids in the widespread distribution of clinically-relevant antimicrobial resistance genes in the built environment, from cities to spacecraft. Together, we present an improved approach to study plasmid biology from fragmented or circularized genomic and metagenomic assemblies.

T16

Plasmid spread from carbapenemase producing *Enterobacter hormaechei* ST79 present on contaminated Dicillin capsules sold in Denmark

Louise Roer [1], Marianne Skov[2,3], Astrid Rasmussen[1], Sanne Løkkegaard Larsen[2], Kat Steinke[2], Anette M. Hammerum[1], Charlotte N. Agergaard[2], Lone J. Porsbo[1], Thomas V. Sydenham[2], Ulrik S. Justesen[2,3] and Henrik Hasman [1]

[1] Statens Serum Institut, Denmark
[2] Odense University Hospital, Denmark
[3] University of Southern Denmark Odense, Denmark

From November 2022 to January 2023, nine closely related carbapenemase producing *Enterobacter hormaechei* ST79 carrying *bla*$_{\text{NDM-5}}$ and *bla*$_{\text{OXA-48}}$ were submitted to Statens Serum Institut (SSI) from patients belonging to four out of five regions in Denmark as part of the national surveillance. Subsequent thorough epidemiological investigations, conducted by the Department of Clinical Microbiology at Odense University Hospital, traced the outbreak to contamination of the surface of several batches of a dicloxacillin medical product (Dicillin® 500 mg capsules). The capsules were administered to approximately 18,500 patients in Denmark between August 2022 and February 2023 [1]. For this reason, all Dicillin products were withdrawn from the Danish market as well as recalled from patients on February 6th 2023.

The carbapenemase genes were located on two different unique plasmids in an *Enterobacter hormaechei* ST79 strain. The two plasmids belonged to IncX3 (for *bla*$_{\text{NDM-5}}$) and IncL (for *bla*$_{\text{OXA-48}}$), respectively.

Several of the patients carrying the outbreak strain were found to also carry other carbapenemase producing *Enterobacteriales* (CPE) with either *bla*$_{\text{NDM-5}}$, *bla*$_{\text{OXA-48}}$, or both genes. Long-read sequencing using Oxford Nanopore Technologies (ONT) of all CPE isolates associated with the outbreak revealed the presence of identical plasmids in all isolates. These two plasmids were used to perform an *in silico* screening against other non-*E. hormaechei* short-read sequencing data originating from all isolates in the Danish national CPE strain collection. This led to identification of several Dicillin-treated patients, who were not carrying the *E. hormaechei* outbreak strain but other *Enterobacteriales* isolates with the outbreak plasmids.

Currently, plasmid-specific Real-Time (RT) PCR protocols are being developed with the aim to perform both retrospective screening of stored wastewater (DNA) samples taken from mid-2021 until the present, as well as to being able to perform prospective screening of the plasmids in stool samples from patients treated with Dicillin.

[1] Agergaard CN, Porsbo LJ, Sydenham TV, Hansen SGK, Steinke K, Larsen SL, Helgason KO, Hansen F, Karstensen KT, Henius AE, Holzknecht BJ, Søes L, Schønning K, Wang M, Ank N, Halldórsdóttir AM, Guðlaugsson Ó, Hammerum AM, Kjerulf A, Kristensen B, Hasman H, Justesen US. Contaminated dicloxacillin capsules as the source of an NDM-5/OXA-48-producing Enterobacter hormaechei ST79 outbreak, Denmark and Iceland, 2022 and 2023. Euro Surveill. 2023, 28:2300108.

T17

Genomic dissection of plasmids driving AMR and hypervirulence convergence in *Klebsiella pneumoniae*

Margaret Lam[1] on behalf of the KlebNET Genomic Surveillance Project (KlebNET-GSP) consortium

[1]*Department of Infectious Diseases, Central Clinical School, Monash University, Melbourne, Australia*

Klebsiella pneumoniae and members from the associated species complex (i.e. KpSC) are leading causes of healthcare-associated infections in clinical settings. They represent a significant public health threat due to rising rates of antimicrobial resistance (AMR), which is often linked to plasmid transmission. Outside of the hospital, some strains of *K. pneumoniae* with hypervirulent properties also cause invasive community-acquired infections. Hypervirulence and AMR have historically been segregated in non-overlapping populations [1, 2] but reports of 'convergent' AMR-virulent strains have become increasingly common in recent years and have been known to cause fatal outbreaks [3]. Like AMR, genes associated with hypervirulence are also predominantly mobilised by plasmids [4]. Plasmid transmission is therefore a significant driver of convergence (i.e. hypervirulent strain acquiring an AMR plasmid or strain with AMR acquiring a virulence plasmid), highlighting the need to monitor the movement of AMR and virulence plasmids in *K. pneumoniae* to detect emergent convergent clones.

Here, we screened a public dataset of n=34,764 *Klebsiella* assemblies (recently uploaded to Pathogenwatch [5]) for AMR-virulence convergence in order to examine the frequency and nature of convergence. More specifically, we used this dataset to explore (i) whether there were particular clones that were overrepresented amongst the convergent population and have had multiple independent plasmid acquisitions giving rise to convergence, and (ii) the population of plasmids and plasmid diversity underpinning convergence. Insights from both of these key overarching questions are important for dissecting the host (i.e. clone) versus plasmid contributions to AMR and virulence convergence in *K. pneumoniae*, and refining surveillance strategies to target 'higher risk' clones and/or plasmids that contribute disproportionately to convergence.

[1] Holt, KE. Wertheim, H. Zadoks, RN. Baker, S. Whitehouse, CA. et al. Proc. Natl. Acad. Sci. USA. **112(27),** E3574-81(2015)
[2] Wyres, KL. Wick, RR. Judd, LM. Froumine, R. Tokolyi, A. et al. PLoS. Genet. **15** (2019)
[3] Lan, P. Jiang, Y. Zhou, J. and Yu, Y. J. Glob. Antimicrob. Resist. **25**, 26-34 (2021)
[4] Lam, MMC. Wyres, KL. Judd, LM. Wick, RR. Jenney, A. et al. Genom. Med. **10**, 77 (2018)
[5] Argimón, S. David, S. Underwood, A. Abrudan, M. Wheeler, NE. et al. Clin. Infect. Dis. **73,** S325-S335 (2021)

T18

The invasion success by antibiotic-resistant plasmid-bacterium associations in human-associated communities is determined by the interplay between intrinsic and environmental factors

Ricardo Leon-Sampedro [1] , Mathilde Boumasmoud [1] , Markus Reichlin [1], Katia R. Pfrunder-Cardozo [1], Adrian Egli [2] and Alex R. Hall [1]

[1] Institute of Integrative Biology, Department of Environmental Systems Science, ETH Zurich, Zurich, Switzerland

[2] Institute for Medical Microbiology, University of Zurich

To address the global crisis of antibiotic resistance, it is critical to understand the spread of plasmid-mediated antibiotic-resistance within complex microbial communities. Unfortunately, we currently lack direct quantitative observations on how resistance emerges in human-associated communities. Moreover, the relative contribution of the factors determining the success of plasmid-bacterium clinical associations at invading human gut microbiome communities remain largely unexplored.

To tackle these knowledge gaps, our study combined replicated anaerobic gut microcosms to track the invasion of clinical *E. coli* strains carrying ESBL and carbapenem-resistance plasmids (IncI-$bla_{\text{CTXM-1}}$, IncF-$bla_{\text{CTXM-14}}$, IncU-$bla_{\text{KPC-2}}$, IncL-$bla_{\text{OXA-48}}$). To set up the microcosms, we obtained anonymous samples from healthy adults, prepared faecal slurry, and then inoculated each microcosm with one of the *E. coli* strains. We conducted the experiment to measure invasion success both in the presence and absence of ampicillin. Additionally, we determined the effects of the presence of the community vs the microcosms environment carrying out supernatant experiments. Furthermore, we identified the interactions between the resident gut microbiome and the invader strain, by analysing the community composition and carrying out competition experiments.

Our findings revealed several important insights. Firstly, in the absence of antibiotics, invasion success varied among the invading antibiotic-resistant *E. coli* strains and among the microbial communities isolated from different individuals. Notably, we found specific interactions with other species, underscoring the significance of these interactions in invading human gut microbiome communities. Additionally, we demonstrate that the intrinsic growth profiles of the invading strains can overcome the interactions with the community successfully invading all microbial communities even in the absence of antibiotics. Overall, these results help to explain the drivers behind invasion success and the emergence of antibiotic resistance in human-associated microbial communities.

T19

Integrons on plasmids: the global genomic epidemiology of *bla*$_{\text{GES-5}}$

William Matlock[1], Liam P Shaw[2*], Nicole Stoesser[1,3,4*], A Sarah Walker[1,3,4*]

[1]*Nuffield Department of Medicine, University of Oxford, Oxford, UK*
[2]*Department of Biology, University of Oxford, Oxford, UK*
[3]*NIHR HPRU in Healthcare-Associated Infection and Antimicrobial Resistance, University of Oxford, Oxford, UK*
[4]*NIHR Oxford Biomedical Research Centre, University of Oxford, Oxford, UK*
[*]*These authors contributed equally.*

Antimicrobial resistance (AMR) gene cassettes comprise of an AMR gene and a short recombination site (*attC*) [1]. Integrons are genetic elements able to capture, excise, and reorder these cassettes, providing 'adaptation on demand' [2], and can be found on both chromosomes and plasmids [1]. Understanding their epidemiology is complex. To date, epidemiology has primarily relied on sequence comparisons to reference databases, such as INTEGRALL [3], which is restricted to known diversity, and due to the rearrangement of gene cassettes, integrons with the same content can have poor alignment scores. Here, we examine integron epidemiology using recent advances in pangenome graphs [4]. As a case study, we look at the clinical resistance gene *bla*$_{\text{GES-5}}$, an integron-associated class A carbapenemase first reported in Greece in 2004 [5] and since observed worldwide [6, 7, 8].

Our dataset consists of all NCBI contigs containing *bla*$_{\text{GES-5}}$ (*n*=431). After deduplication, we identified *n*=104 distinct *bla*$_{\text{GES-5}}$ contigs isolated between 2006 and 2022, representing all global ISO regions (*n*=3 Africa, *n*=17 Americas, *n*=36 Asia, *n*=39 Europe, *n*=6 Oceania) and *n*=10 genera, of which 61% (63/104) were from *Pseudomonas*. By clustering the sequence diversity in the flanking regions around the gene, we could identify different characteristic integron structures. We found that 14/104 contigs contained plasmid replicons from diverse plasmid families (*n*=5 IncP6, *n*=5 IncQ2, *n*=3 IncQ1, *n*=1 IncL/M). Our approach could cluster structures on short contigs that could not be identified as plasmid or chromosomal with classified replicons. In total, 36% (37/104) contigs were annotated for an integrase, and of these, 73% (27/37) had an *attI* site and at least one *attC* site, indicating a complete integron.

Our results suggest that *bla*$_{\text{GES-5}}$-associated integrons on plasmids are different to those on chromosomes. Chromosomal integrons were mostly found in *P. aeruginosa* ST235, which had a consistent gene cassette content and order. Structural variants emerged when IS*110*-familly insertion sequences disrupted *attC* sites, which might immobilise the gene cassettes and explain the conserved integron structure, despite the presence of *intI1* integrase promoters. The plasmid-associated integrons were more diverse in their gene cassette content and order, which could be an indication of greater integrase activity and 'shuffling' of integrons on plasmids. Pangenome approaches offer a way to explore the interaction between different mobile genetic elements and structural diversity.

[1] Partridge, Sally R., et al. "Gene cassettes and cassette arrays in mobile resistance integrons." *FEMS microbiology reviews*33.4 (2009): 757-784.
[2] Souque, Celia, Jose Antonio Escudero, and R. Craig MacLean. "Integron activity accelerates the evolution of antibiotic resistance." *Elife* 10 (2021): e62474.
[3] Moura, Alexandra, et al. "INTEGRALL: a database and search engine for integrons, integrases and gene cassettes." *Bioinformatics* 25.8 (2009): 1096-1098.
[4] Noll, Nicholas, et al. "Pangraph: scalable bacterial pan-genome graph construction." *bioRxiv* (2022): 2022-02.
[5] Vourli, Sofia, et al. "Novel GES/IBC extended-spectrum β-lactamase variants with carbapenemase activity in clinical enterobacteria." *FEMS microbiology letters* 234.2 (2006): 209-231.
[6] Doumith, Michel, et al. "Genomic characterization of carbapenem-non-susceptible Pseudomonas aeruginosa clinical isolates from Saudi Arabia revealed a global dissemination of GES-5-producing ST235 and VIM-2-producing ST233 sub-lineages." *Frontiers in Microbiology* 12 (2022): 4155.
[7] Ellington, Matthew J., et al. "A multispecies cluster of GES-5 carbapenemase–producing Enterobacterales linked by a geographically disseminated plasmid." *Clinical Infectious Diseases* 71.10 (2020): 2553-2560.
[8] Kanayama, A., et al. "Successful control of an outbreak of GES-5 extended-spectrum β-lactamase-producing Pseudomonas aeruginosa in a long-term care facility in Japan." *Journal of Hospital Infection* 93.1 (2016): 35-41.

T20

Transference of antibiotic resistance genes through marine conjugative plasmids

Juan Manuel Medina-Méndez [1], Santiago Redondo-Salvo [1], María Pilar Barcillán-García [1], Raúl Fernández-López [1] and Fernando de la Cruz [1]

[1] *Institute of Biomedicine and Biotechnology of Cantabria (IBBTEC), Santander, Spain*

Antibiotic resistance genes (ARGs) are one of the most challenging contaminants of emerging concern. Instead of being directly produced by human activity, ARGs emerge as consequence of antibiotic use in clinical settings, and residual antibiotic contamination. ARGs are disseminated among bacteria of diverse taxonomy by horizontal gene transfer (HGT) through conjugative plasmids, increasing their survival rate. Recent studies have revealed the existence of marine plasmids (MPs) with global distribution and wide host range [1]. These MPs can transport ARGs over intercontinental distances, and they could reintroduce them into human food chains through the consumption of seafood, just like some algal degradative genes found in the human gut microbiota [2].

The relaxase (RLX) is the only common protein in the mobilization machinery of mobilizable plasmids. Its function is to initiate bacterial conjugation, and it allows classifying conjugative systems into MOB families [3]. In this study we have used the RLX as a molecular target to explore the distribution and abundance of MPs. For this, we have extracted and classified the complete plasmids of RefSeq200, as well as those MPs also annotated in the MarRef6 database. In addition, we have quantified the RLXs and the ARGs in two groups of marine metagenomic samples (MGs) obtained during the GEOTRACES expedition and from samplings carried out in two oceanic stations, and we have compared the results with their abundance in a group of human fecal MGs.

The results reflect that the MOB proportions of the MPs are maintained between the databases and the marine MGs. However, RLXs and ARGs are scarce in the sea. This leads us to believe that bacterial conjugation is a rare HGT phenomenon in the sea, and that the ARGs could be moved by a specific group of specialized MPs.

[1] Petersen, Jörn, *et al.* A marine plasmid hitchhiking vast phylogenetic and geographic distances. *Proceedings of the National Academy of Sciences*, vol. 116, no. 41, pp. 20568-73 (2019).
[2] Pudlo, Nicholas A., *et al.* Diverse events have transferred genes for edible seaweed digestion from marine to human gut bacteria. *Cell Host & Microbe*, vol. 30, no. 3, pp. 314-328.e11 (2022).
[3] Garcillán-Barcia, María Pilar, *et al.* The diversity of conjugative relaxases and its application in plasmid classification. *FEMS Microbiology Reviews*, vol. 33, no. 3, pp. 657–87. (2009).

T21

Capturing microbial genome diversity with pangenome graphs: the case of E. coli ST131

Marco Molari[1,2], **Nicholas Noll**[3], **Liam P. Shaw**[4] **and Richard A. Neher**[1,2]

[1] *Swiss Institute of Bioinformatics, Basel, Switzerland*
[2] *Biozentrum, University of Basel, Basel, Switzerland*
[3] *Kavli Institute for Theoretical Physics, University of California, Santa Barbara*
[4] *Department of Biology, University of Oxford, Oxford.*

Capturing the genetic diversity of microbial populations is a challenging task. Mutations, frequent gene movement and recombination generate both small-scale nucleotide-level changes (SNPs, small indels...), but also large-scale structural rearrangements and differences in accessory genome. Traditional methods, based uniquely on comparison of core genetic sequence, or on presence/absence of particular accessory genes, struggle to fully capture this complexity.

To this end, in the lab we have been working on a pangenome-graph representation for a set of microbial genomes [1] that encompasses all of these different layers. In this representation *blocks* encode homologous sequence alignments, and genomes of single isolates are represented as oriented *paths* through blocks.

In addition to presenting pangenome graphs, in this talk I will show preliminary results on their use for the study of microbial genome evolution, focusing on *E. coli* ST131. This multidrug-resistant clade recently evolved under antibiotic selection pressure, and includes isolates that are extremely similar on their core genome, but can differ in hundreds of accessory genes. For this dataset the graph representation can help us disentangle and relate the different layers of diversity.

[1] N. Noll, M. Molari, L. P. Shaw and R. A. Neher. (2023) *PanGraph: scalable bacterial pangenome graph construction.* Microbial Genomics (in press).

T22

Abstract for Contributed Talk

Investigation of the extent of clonal and multiple acquisition in OXA-48 plasmids in UK hospitals

Fan Grayson[1], Toby Nonnenmacher[1], Leo Loman[1] and Alice Ledda[1,2]

[1]*UK Health Security Agency, London, UK.*

[2]*Imperial College London*

Background:

Transfer of anti-microbial resistance (AMR) mechanisms has been occurring within both community and hospital environments. To understand the extent of prevalence of different methods of conjugation, the use of bioinformatic techniques have been employed to investigate common ancestries and the manner in which mutations have occurred. Understanding AMR is a high priority for the UK Health Security Agency (UKHSA).

Methods:

An interdisciplinary working group at the UKHSA spanning genomics and data science expertise have undertaken an analysis of DNA sequence data of plasmids containing the OXA-48 gene. Our dataset included 260 samples at a short read level, which span 120 patients across approximately 30 hospitals. These samples span multiple bacteria including E. Coli and Klebsiella.

In order to make use of the dataset, we have performed genome assembly and alignment using methods such as hybrid De-Novo and referenced based assembly. We constructed phylogenetic trees in order to analyse the potential evolutionary path of the plasmid and determine whether horizontal or vertical transfer has taken place. We analysed the proportionality between genetic distance in units of mutation and distance based on time

Genome assembly and alignment was performed from short reads of plasmids containing the OXA-48 gene, in a dataset spanning a wide geographical area (multiple hospitals across the UK). We constructed a phylogenetic tree, to determine the degree to which horizontal or vertical conjugation have occurred.

Results:

Preliminary results indicate that we observe greater multiple acquisition in our sample, than clonal acquisition. There were indications of horizontal transfer in the data set. We also saw that the OXA-48 gene was conserved perfectly in almost all of the samples whereas the transposases either side were conserved less well.

Discussion:

The results of the work give insight into the nature of transmission of AMR within UK hospitals (in a dataset spanning a broad geography). This work will be used to better understand community and

hospital transfer as well as inform UKHSA’s recommendations on tailoring policy driven interventions.

T23

Detecting AMR plasmid variants in *Shigella sonnei*

Sally Partridge [1,2,3,4], Jenny Draper [3,5,6], Grace Blackwell [3,5,6], Elena Martinez [3,5,6], Eby Sim [3,4,6], Indy Sandaradura [2,3,5,6], Andrew Ginn [3,5], Vitali Sintchenko [1,3,4,5,6], and Jon Iredell [1,2,3,4,6]

[1]The Westmead Institute for Medical Research, Westmead, NSW, Australia
[2]Western Sydney Local Health District, Westmead, NSW, Australia
[3]Faculty of Medicine and Health, The University of Sydney, Sydney, NSW, Australia
[4]Sydney Institute for Infectious Diseases, The University of Sydney, Sydney, NSW, Australia.
[5]Institute of Clinical Pathology and Medical Research, New South Wales Health Pathology, NSW, Australia.
[6]Centre for Infectious Diseases and Microbiology-Public Health, Westmead Hospital, Sydney, NSW, Australia.

Shigella spp. are a major cause of severe diarrhoeal disease worldwide, with sexual transmission responsible for increased spread of antibiotic resistant strains in developed countries [1]. Illumina sequencing of 600 *Shigella sonnei* isolates recovered from clinical samples from 2017-2020 in New South Wales, Australia, revealed different genotypes [2] and clusters (≤5 single nucleotide variants, SNV, in the core genome). Many isolates (70%) carry the *mph*(A) gene, mostly together with *erm*(B), both conferring resistance to macrolides, and 30% have a $bla_{\text{CTX-M}}$ gene (resistance to third generation cephalosporins), mostly $bla_{\text{CTX-M-27}}$. Long-read sequencing (Oxford Nanopore, ONT, and/or PacBio HiFi) of 20 isolates identified FII plasmids carrying *mph*(A), *erm*(B) and/or $bla_{\text{CTX-M-27}}$, related to those reported previously in *Shigella,* e.g., [3]. Alignment of ONT/Illumina hybrid assemblies and mapping of short reads identified some differences as errors that could not be fully resolved by automated polishing, while HiFi sequences had very few errors. After correcting errors, we identified genuine SNV, and insertions, deletions and rearrangements involving multi-resistance regions (MRR).
To survey the Illumina data from all 600 genomes, we compiled target sequences covering distinctive boundaries, e.g., between MRR modules, between plasmid backbones and MRR or other insertions and both versions of SNV, for use in a *k-mer* counting approach [4]. The 20 isolates with assembled FII plasmids gave the expected *k-mer* patterns. For selected isolates, results were consistent with mapping of Illumina contigs, suggesting that this method can reliably identify plasmid variants. Overall, *k-mer* patterns were consistent within each cluster, sometimes across several clusters, but with evidence of plasmid evolution within some clusters, and four distinct plasmid variants were identified in one cluster. Our findings appear to contrast with previous reports of spread of the same FII AMR plasmid between *Shigella* species and lineages [3]. This may be partly due to differences in perspective in deciding when plasmids should be considered 'the same' or 'different', highlighting the need for a standardised approach in surveillance of plasmid outbreaks.

[1] H. O'Flanagan, M. Siddiq, C. Llewellyn, D. Richardson, Int. J. STD AIDS **34**, 374 (2023).
[2] J. Hawkey, K. Paranagama, K.S. Baker, R.J. Bengtsson, F.X. Weill, N.R. Thomson, S. Baker, L. Cerdeira, Z. Iqbal, M. Hunt, D.J. Ingle, T.J. Dallman, C. Jenkins, D.A. Williamson, K.E. Holt, Nat Commun **12**, 2684 (2018).
[3] K.S. Baker, T.J. Dallman, N. Field, T. Childs, H. Mitchell, M. Day, F.X. Weill, S. Lefèvre, M. Tourdjman, G. Hughes, C. Jenkins, N. Thomson, Nat Commun **9**, 1462 (2018).
[4] G. Marçais, C. Kingsford, Bioinformatics. 27, 764 (2011).

T24

Abstract template for the workshop "Plasmids as vehicles of AMR spread"

Arancha Peñil-Celis[1], Kaitlin A Tagg[2], Hattie E Webb[2], Santiago Redondo-Salvo[1,5], Louise Francois Watkins[2], Luis Vielva[6], Chelsey Griffin[2,3], Justin Y Kim[2,4], Jason P Folster[2], M Pilar Garcillan-Barcia[1], Fernando de la Cruz[1]

[1]Instituto de Biomedicina y Biotecnología de Cantabria, (CSIC, Universidad de Cantabria), Santander, Spain

[2]Division of Foodborne, Waterborne, and Environmental Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, United States

[3]Oak Ridge Institute for Science and Education (ORISE), Oak Ridge, Tennessee, United States

[4]ASRT, Inc., Suwanee, Georgia, United States

[5]Biomar Microbial Technologies, León, Spain

[6]Departamento de Ingeniería de las Comunicaciones, Universidad de Cantabria, Santander, Spain

Public health genomic surveillance systems traditionally focus on chromosomal core loci to infer molecular epidemiological relationships, relying on vertical evolution, that is, homology-by-descent. However, the accessory genome, consisting of plasmids and other mobile genetic elements, plays a crucial role in bacterial evolution through horizontal gene transfer, facilitating rapid adaptation in response to environmental selection pressures, such as antimicrobials. By incorporating homology in the accessory genome, homology-by-admixture, we can gain valuable molecular epidemiological insights. In this study, we employed the Jaccard Index (JI) to measure the pangenomic relatedness in *Salmonella enterica* serotype Typhi (Typhi), a globally significant pathogen. By implementing a reticulate JI network, we graphically depicted both homology-by-descent and homology-by-admixture. The JI Network Analysis unveiled a structured pangenome in Typhi, and revealed that its short-term evolution is defined by the loss and gain of accessory genome elements. Genomes with a multidrug-resistance profile were confined to three JI clusters, each containing a unique Plasmid Taxonomic Unit, while extremely drug-resistance profile emerged by the acquisition of a $bla_{CTX\text{-}M\text{-}15}$-encoding plasmid and subsequent independent integration events in the chromosome. This approach complements existing phylogenetic methods by offering an additional molecular dimension for clonal serovars such as Typhi, and may prove useful for other *Salmonella* serovars.

T25

IncC plasmids in the emergent genotype ST213 of S*almonella* Typhimurium: Phenotypic analyses of plasmid-cured mutant strains and comparative genomics.

Marco Arturo Pérez Sánchez[1], Isela Serrano-Fujarte[1] and Jose L. Puente[1]

[1]Departamento de Microbiología Molecular, Instituto de Biotecnología, Universidad Nacional Autónoma de México.

Salmonella enterica serovar Typhimurium is one of the most frequently isolated foodborne pathogens, capable of infecting a wide variety of animals, including mammals, reptiles, and birds [1]. In humans commonly causes self-limiting enteric disease but sometimes leads to systemic infection. In recent years, the emergence of strains from different genotypes associated with multidrug resistance, invasive infections, and severe gastroenteritis has increased [2].

During an epidemiological surveillance program in Mexico, the emergent genotype ST213 was isolated from retail meat, as well as from healthy and ill individuals, including some cases of systemic infection. Most ST213 strains show high levels of antimicrobial resistance encoded in an IncC plasmid, and all lack the characteristic *Salmonella* virulence plasmid (pSTV). Strains of this genotype have predominantly been isolated in North America, suggesting that they are replacing the ST19 genotype, considered the founder genotype of Typhimurium globally [3].

In the ST213 strains from México, two types of IncC plasmids were identified with a specific geographic distribution. Type I plasmids carry the *cmy-2* gene and are the largest (>150 kb) of the two types and the most abundant in the southern region, while Type II plasmids lack the *cmy-2* gene and are mainly found in strains isolated in the northern region [4]. It has not been precisely characterized which other genes or segments of the Type I plasmids are absent in the Type II plasmids or if they possess other distinctive features. Several studies have proposed "backbones" for the IncC plasmids deposited in GenBank, in which the core genes and the major hotspots of integration of mobilizable genetic elements were identified. Nevertheless, the genetic structure and evolutionary characteristics of IncC ST213 plasmids remain unclear [5].

ST213 strains represent a public health risk, and carrying the IncC plasmids could be a key factor contributing to their ecological success. Therefore, this work aims to eliminate, using molecular biology strategies, the IncC plasmid in two representative ST213 strains isolated from patients with systemic infection. Subsequently, a comparison will be made between the parental and IncC-cured strains, evaluating pathogenesis and bacterial survival-related phenotypes through cell culture and biofilm formation assays. Furthermore, a comparative genomics analysis will be conducted on IncC plasmids from ST213 genomes available in public databases to construct their pangenome and identify genes that may play a significant role in the bacterial physiology of these strains.

The significance of this research is that it provides insights into the evolutionary dynamics of IncC plasmids and sheds light on how the proliferation of these plasmids has contributed to the emergence of the ST213 genotype.

[1] A. Fabrega and J. Vila, *Clin Microbiol Rev* **26**, 308-341 (2013).
[2] J. Gilchrist and C. MacLennan , *EcoSal Plus* **8**, 0007-2018 (2019).
[3] B. Zaidi *et al.*, *Emerg Infect Dis* **14**, 429-435 (2008).
[4] M. Wiesner *et al.*, *BMC Microbiology* **11**, 9 (2011)
[5] F. Zhang *et al.*, *Front Microbiol* **13**, 1045314 (2022)

T26

Genomic Epidemiologic Investigation of a Hospital Outbreak of NDM-5-Producing Gram-Negative Enterobacterales Infections

Nathan J. Raabe, MPH[1,2,3] , Abby Valek, MPH, CIC[4] , Marissa P. Griffith, BS[1,2] , Emma Mills, BS[2] , Kady Waggle, BS[2,3] , Vatsala Rangachar Srinivasa, MPH[1,2] , Ashley Ayres, MBA, BS, CIC[4] , Claire Bradford, MPH[4] , Hannah Creager, PhD, D(ABMM)[5,6] , Lora Pless, PhD[1,2] , Alexander J. Sundermann, DrPH, MPH[1,2] , Daria Van Tyne, PhD[2] , Graham Snyder, MD, MS[2,4] , Lee H. Harrison, MD[1,2,3]

[1]*Microbial Genomic Epidemiology Laboratory, Center for Genomic Epidemiology, University of Pittsburgh, Pittsburgh, Pennsylvania, USA.*
[2]*Division of Infectious Diseases, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA.*
[3]*Department of Epidemiology, School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania, USA.*
[4]*Department of Infection Control and Hospital Epidemiology, UPMC Presbyterian, Pittsburgh, Pennsylvania, USA.*
[5]*Department of Pathology, University of Pittsburgh, Pittsburgh, Pennsylvania, USA.*
[6]*Lab Service Center, UPMC Presybyterian, Pittsburgh, Pennsylvania, USA.*

Background: New Delhi Metallo-β-lactamase (NDM) is an emergent carbapenem resistance mechanism associated with high mortality and limited antimicrobial treatment options. Because the *bla*$_{NDM}$ resistance gene is often carried on plasmids, traditional infection prevention and control (IP&C) surveillance methods using species- and antimicrobial resistance phenotype-based reactive whole genome sequencing (WGS) may not detect plasmid transfer in multispecies outbreaks.

Methods: Initial outbreak detection of NDM-producers identified at an acute care hospital occurred via traditional IP&C methods and was supplemented by real-time WGS surveillance. WGS was performed weekly using the Illumina platform. To resolve NDM-encoding plasmids, we performed long-read Nanopore sequencing and constructed hybrid assemblies using Illumina and Nanopore sequencing data. Reports of relatedness between NDM-producing organisms and reactive WGS for suspected outbreaks were shared with the IP&C team for assessment and intervention.

Results: We observed a multispecies outbreak of NDM-5-producing Enterobacterales isolated from 15 patients between February 2021 and February 2023. The 19 clinical and surveillance isolates sequenced included seven distinct bacterial species and each encoded the same NDM-5 plasmid, which showed high homology to NDM plasmids previously observed in Asia. WGS surveillance and epidemiologic investigation characterized ten horizontal plasmid transfer events and six bacterial transmission events between patients housed in varying hospital units. Transmission prevention focused on enhanced observation and adherence to basic prevention measures.

Conclusions: Our investigation revealed a complex, multispecies outbreak of NDM that involved multiple plasmid transfer and bacterial transmission events, increasing the complexity of outbreak identification and transmission prevention. Our investigation highlights the utility of combining traditional IP&C and prospective genomic methods in identifying and containing plasmid-associated outbreaks.

T27

RoundHound: detecting plasmid transmission from short-read datasets

Leah W Roberts[1,2], Leandro Lima[1], Daria Frolova[1], and Zamin Iqbal[1]

1. European Molecular Biology Laboratory, European Bioinformatics Institute (EMBL-EBI), Hinxton, United Kingdom (UK)

2. Department of Medicine, University of Cambridge, Cambridge, United Kingdom (UK)

The phylogenetic analysis of bacterial genomes to detect outbreaks is a mature field. However clonal spread of successful strains is only one type of outbreak; the other is caused by the spread of plasmids (small DNA molecules that can transfer genes between bacterial cells) moving across different strains or even species. These outbreaks are particularly important to spot in hospital settings, as plasmids are common vectors of antimicrobial resistance (AMR). However, most genomic surveillance data is produced using short-read sequencing methods that are unable to fully resolve plasmids (due to repetitive DNA regions that confound short-read assemblers). There is also a lack of methods to compare plasmids and decide whether they are closely related, as they evolve as much by rearrangement and gene gain/loss as by mutation. To date, several tools have been developed to identify plasmids from short-read data, but none have been designed to specifically address plasmid transmission.

Here we present RoundHound, a tool designed to detect plasmid transmission directly from short-read sequencing data. We first construct a plasmid reference database using all plasmids from PLSDB (~32,000). A plasmid pangenome is created by annotating (Bakta) and clustering (mmseqs2) all plasmid genes, which are then stored as pan-genome reference graphs (PRGs). A plasmid network is also constructed using a combination of gene-jaccard and rearrangement/indel distances, and then divided into communities using the asynchronous label propagation algorithm. To query each sample, short-reads are mapped to the plasmid pan-genome to identify gene presence/absence and variants, which is used to determine the presence/absence of plasmids and their associated community. Multiple samples that hit the same community are then compared to each other to determine the likelihood of having a shared plasmid based on shared genes and SNP distances within the community pan-genome. RoundHound uses the pan-genome variant caller Pandora for mapping and variant detection, and takes on average 3 minutes per sample to run.

We tested RoundHound using two short-read datasets with known plasmid transmission validated with long-read sequencing (Roberts et al., 2022, Hawkey et al., 2022). In both datasets, we had a recall of >95% and precision of >97% when detecting plasmid transmission between bacterial samples. RoundHound is a powerful tool for enhancing surveillance of plasmid transmission in vulnerable settings (such as healthcare) and could be used to strategically select samples for long-read sequencing and further plasmid analysis.

T28

The role of intracellular competition in the fixation of novel plasmids

Fernando Rossine [1] , Carlos Sanchez [1] , and Michael Baym [1]

[1](Presenting author underlined) Harvard Medical School

Ever since the widespread adoption of antibiotics, the fast rise of infections caused by antibiotic resistant bacteria has become a major global health concern. Most frequently, the genes that endow bacteria with antibiotic resistance are carried in plasmids—self-replicating genetic elements— rather than in the bacterial chromosome. Therefore, it is a priority to understand the dynamics of emergence and loss of novel plasmids carrying antibiotic resistance genes (ARGs). An important feature distinguishing plasmids from the chromosome is that, because they are oftentimes present as multiple copies per cell, plasmid copies intracellularly compete with each other for replication. An exciting possibility is that this intracellular competition might be limiting the emergence and even promoting the loss of plasmids carrying ARGs. However, direct measurements of intracellular plasmid fitnesses have never been achieved. Here, I develop a method to measure the relative intracellular fitness of plasmids carrying different genes and promoters. This method is based on the construction of plasmid dimers that can be intracellularly dissociated into monomers, setting up competition experiments. Second, I will apply this method to show that transcription-replication conflicts can mediate intracellular competition, favoring the fixation of “silent" plasmids, which could potentially oppose the maintenance of ARGs.

T29

Anti-Defense Systems on Plasmids

Bruria Samuel[1], David Burstein[1]

[1]The Shmunis School of Biomedicine and Cancer Research, George S. Wise Faculty of Life Sciences, Tel-Aviv University

Plasmids and other conjugative elements facilitate the horizontal transfer of genes encoding various functions, including antibiotic resistance and virulence factors. In order to establish in recipient bacteria, the conjugative elements must overcome different defense mechanisms, such as CRISPR-Cas systems, restriction enzymes, and SOS response. We explored an extensive set of conjugative elements from diverse environments and discovered that anti-defense systems, encoding anti-CRISPR, anti-restriction, and anti-SOS, are highly overrepresented in the leading region of plasmids. This largely uncharacterized region is the first to enter recipient cells during conjugation. Further, we found that anti-defense systems in the leading region tend to cluster into "islands" that contain various combinations of anti-defense and anti-defense-related proteins. Early expression of the leading regions' genes, even before the transfer is complete, is enabled by special single-stranded promoters, which are prevalent in the islands we identified. Our results suggest that anti-defense islands on conjugative elements are expressed upon entry, promoting rapid protection against host defense systems. Uncovering these islands and characterizing them may considerably improve our understanding of the repertoire of anti-defense genes, plasmid dissemination, and the intricate co-evolution of plasmids and their hosts.

T30

Identifying plasmid transconjugants in the rare biosphere of the barley rhizosphere using Hi-C and target enrichment

Salvador Castaneda-Barba[1,2,3] , Eva M. Top[1,2,3] , and Thibault Stalder [1,3]

[1] Institute of Interdisciplinary Data Sciences
[2] Bioinformatics and Computational Biology Graduate program
[3] Department of Biological Sciences, University of Idaho, Moscow, ID 83844 USA

Many plasmid-borne antibiotic-resistance genes found in pathogens originate from bacteria living in human, animal, and environmental habitats. However, the ecological and evolutionary trajectories leading these antimicrobial-resistance (AMR) plasmids to spread within and between habitats remain poorly understood. As a result, there is a major gap in our understanding of AMR emergence and global spread. Interfaces where microbes from different habitats overlap, such as crops grown in manure-amended soil, are suspected to be hotspots for the transfer of AMR plasmids from one habitat to another. Nevertheless, identifying the new hosts that acquire an AMR plasmid in a complex microbial community such as soil is challenging. Transfer frequencies are often too low to be detected *in situ* by metagenomics, and most metagenomic approaches are limited in their ability to identify new hosts of a plasmid. Here, we present a study that utilizes a modified Hi-C proximity ligation approach to monitor and identify the spread of an AMR plasmid introduced via manure in the rhizosphere of barley in manured amended soil.

We first mixed a realistic amount of *Escherichia coli* carrying the AMR plasmid pB10 with manure and subsequently added the mixture to agricultural soil. In accordance with agricultural practices, we waited 30 days before planting barley seeds. To identify if plasmid transfer to soil bacteria had occurred, we combined Hi-C with a target enrichment approach aimed at enriching for pB10 sequences. This allows us to detect rare plasmid transfer events and identify new hosts of pB10. Using this new approach, our preliminary data suggest that pB10 transferred to rhizosphere bacteria by 10 days post-seeding. However, we did not detect new hosts carrying pB10 at subsequent time points. This suggests that despite plasmid transfer, those new transconjugants may have been extinct, or below the detection limit. Importantly, we found that using realistic agricultural practice, *E. coli* was able to transfer an AMR plasmid to a new host up to 40 days after inoculation into soil. The identification of the host that received the plasmid is currently under investigation.

T31

Background

Carbapenemase-producing Enterobacterales (CPE) are challenging in the healthcare setting, with resistance to multiple classes of antibiotics and a high associated mortality. The incidence of CPE is rising globally, despite enhanced awareness and control efforts. This study describes an investigation of the emergence of IMP-encoding CPE amongst diverse Enterobacterales species between 2016 and 2019 in patients across a London regional hospital network.

Methods

We carried out a network analysis of patient pathways, using electronic health records, to identify contacts between IMP-encoding CPE positive patients. Genomes of IMP-encoding CPE isolates were analysed and overlayed with patient contacts to imply potential transmission events.

Results

Genomic analysis of 84 Enterobacterales isolates revealed diverse species (predominantly *Klebsiella* spp, *Enterobacter* spp, *E. coli*), of which 86% (72/84) harboured an IncHI2 plasmid, which carried both bla_{IMP} and the mobile colistin resistance gene *mcr-9* (68/72). Phylogenetic analysis of IncHI2 plasmids identified three lineages which showed significant association with patient contact and movements between four hospital sites and across medical specialities, which had been missed on initial investigations.

Conclusions

Combined, our patient network and plasmid analyses demonstrate an interspecies, plasmid-mediated outbreak of bla_{IMP}CPE, which remained unidentified during standard microbiology and infection control investigations. With DNA sequencing technologies and multi-modal data incorporation, the outbreak investigation approach proposed here provides a framework for real-time identification of key factors causing pathogen spread. Analysing outbreaks at the plasmid level reveals that resistance may be wider spread than suspected, allowing more targeted interventions to stop the transmission of resistance within hospital networks.

T32

Evolutionary interplay of chromosomal and plasmid-borne compensatory mutations

Christopher Witzany [1] and Sebastian Bonhoeffer [1]

[1] *Institute of Integrative Biology, ETH Zürich, Zürich, Switzerland*

The rise of antimicrobial resistance (AMR) threatens the effectiveness of essential medical interventions. Conjugative plasmids drive the spread of AMR within and across bacterial communities and lineages. While conferring potentially beneficial traits, such as AMR, plasmids also impose fitness costs on their bacterial hosts. However, empirical evidence shows that these fitness costs are rapidly mitigated by compensatory evolution. Compensatory mutations can occur on the chromosome and the plasmid – with a pioneering modeling study showing superiority of plasmid-borne compensatory mutations [1]. Plasmid-borne compensation confers greater benefits, as they have the advantage of spreading horizontally in addition to vertically. However, empirical data frequently shows compensation to occur only on the chromosome or in both locations simultaneously [2-5]. This suggests that chromosomal compensation might be more likely to emerge and that plasmid-borne and chromosomal mutations interplay. While chromosomal compensation can ameliorate costs of multiple plasmids for a single host, plasmid-borne compensation can expand the plasmids host range [3, 4]. Understanding when and how compensatory mutations emerge in specific locations is hence key to predicting spread and persistence of AMR plasmids across bacterial lineages. We use mathematical models and stochastic simulations to investigate how chromosomal and plasmid-borne compensatory mutations emerge, interact and what ultimately determines whether compensation on the chromosome, plasmid, or both prevails. We find that an existing compensatory mutation, can only be superseded by a new mutation located elsewhere if it confers a greater benefit or a higher conjugation rate. However, chromosomal and plasmid-borne compensation can accumulate in the same host cell due to conjugation even in the absence of an additional fitness benefit.

References

[1] M. Zwanzig, E. Harrison, M. A. Brockhurst, J. P. J. Hall, T. U. Berendonk and U. Berger. mSystems (2019) doi: 10.1128/mSystems.00186-18

[2] J. P. J. Hall, R.C.T. Wright, D. Guymer, E. Harrison and M. A. Brockhurst. Microbiology (2020) doi: 10.1099/mic.0.000862

[3] C. Dahlberg and L. Chao, Genetics (2003) doi: 10.1093/genetics/165.4.1641

[4] W. Loftie-Eaton, K. Bashford, H. Quinn, K. Dong, J. Millstein, S. Hunter, M. K. Thomason, H. Merrikh, J. M. Ponciano and E. M. Top. Nature Ecology & Evolution (2017) doi: 10.1038/s41559-017-0243-2

[5] H. Jordt, T. Stalder, O. Kosterlitz, J. M. Ponciano, E. M. Top, and B. Kerr. Nature Ecology & Evolution (2020) doi: 10.1038/s41559-020-1170-1