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P01

Global map of evolutionary dependencies between antibiotic resistance and virulence genes in *E. coli*

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Genes conferring antibiotic resistance or virulence phenotypes frequently undergo horizontal gene transfer in bacteria, contributing to the emergence of new multidrug resistant pathogenic variants, so called "superbacteria". Mounting evidence indicates that pre-existing genome content variations influence the successful acquisition of such genes. However, the underlying evolutionary dependencies among specific genes, i.e. when one gene facilitates or hinders the acquisition of a second gene, remain poorly understood. This gap of knowledge impedes our ability to forecast the dissemination of resistance genes and the emergence of superbacteria. Here we chart a high-resolution map of evolutionary dependencies between resistance and virulence genes by phylogenetic analysis of more than 10,000 *Escherichia coli* genomes. By reconstructing the gain and loss events of each gene along the phylogeny, we identify pairs of genes for which the presence of one gene significantly influences the gain of the other gene. With the dependency networks in hand we aim to answer questions of the field like: (i) Is there a general negative correlation between bacterial virulence and resistance levels? (ii) Do we find clades that have the potential to become both multi-resistant and virulent? (iii) Is the genome introgression of certain resistance and virulence genes influenced by genetic background or by other factors (*e.g.* fitness effect, habitat, origin of the gene)?

P02

Tanin rich tree leaves as feed additives to minimise the use of antimicrobial drugs in Sheep of Reasi district of India

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Background : Over the last few years, the dietary role of tannins is receiving increasing interest as they may reduce the number of gastrointestinal parasites in mammals (Athanasiadou *et al*., 2000; Butter *et al*., 2002; Min *et al.*, 2005) and birds (Marzoni *et al.*, 2005). Present study was carried out to assess the effect of locally avalable agroforestry tree leaves in hilly areas of Jammu and Kashmir, India on production potential and parasitic control of sheep. Data was collected on feed intake, weight gain, fecal egg counts and overall body score in 60 sheep during an experimental period of 90 days. The result showed use of agroforestry tree leaves as leaf meal mixture of guava (*Psidium guajava*): neem (*Azadirachta indica*): jamun (*Syzygium cuminii*): mango (*Magniferra indica*) in 1:1:1:1 proportion at 1.5% level resulted in significant (P<0.05%) increased weight gain, improved feed conversion ratio and improved overall body score and significantly (P<0.05) decreased fecal egg count as compared to rest of treatments. Thus it can be concluded agroforest tree leaf Meal mixtures of guava (Psidium guajava): neem (Azadirachta indica): jamun (Syzygium cuminii): mango (Magniferra indica) is an alternative feed additives for improving production potential sheep.

Material and Methods: Present experiment was carried out to study the effect of different agroforestry tree leaf meal mixtures on the production performances of sheep in Himalayan region of Jammu and Kashmir, India. 60 sheep were randomly divided into three groups (T1, T2 and T3) of 20 animals in each group in a completely randomized block design for a period of 3 months. The locally available agroforestry trees like, guava (Psidium guajava), neem (Azadirachta indica), jamun (Syzygium cuminii) and mango (Magniferra indica) found in hilly areas of Jammu and Kashmir were used to prepared leaf meal mixtures in 1:1:1:1 proportion and used at different inclusion levels (T1=0 %, T2=0.5%, T3=1.5%). Three iso-caloric and iso-nitrogenous diets were formulated with inclusion of agroforestry leaf meal mixtures at 0%, 0.5%, 1.5% for T1, T2, T3 respectively. Experiment lasted for 90 days. Data regarding daily feed intake, adult body

weight, Average body weight gain, FCR, body score and fecal egg count were recorded and results were subjected to one way ANOVA accordingly.

Results: Study shows there is no significant change in dry matter intake of sheep of T1, T2 and T3 groups. Daily body weight gain, average final body weight, FCR and overall body score were significantly increased in T3 group as compared to T1, T2. Fecal egg count was significantly ($P<0.001$) decreased in T3 groups as compared to T1 and T2. Overall, the use of leaf meal mixture @ 1.5% improved the overall body score as compared to controlled group. Leaf meal mixture prepared from guava (Psidium guajava), neem (Azadirachta indica), jamun (Syzygium cuminii) and mango (Magniferra indica) in 1:1:1:1 proportion when used at 1.5% in the ration significantly increased the overall production performances.

Conclusion: The use of agro forest leaf meal mixtures appear as alternative to the use of antimicrobial growth promoter factors. These natural products do not leave residues. Also, agro forest leaf meal mixtures contain photochemical substances with many bioactive principles that would have fewer chances to induce resistance in microorganisms.

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P03

Long reads sequencing to support outbreak investigations in the healthcare setting

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In 2022, a sudden increase in the number of infections sustained by VIM+ Enterobacterales prompted an outbreak investigation in a Northern Italy hospital. A total of 27 strains, 10 collected from the hospital environment and 17 clinical isolates, underwent cluster analysis through Whole Genome Sequencing (WGS).

WGS analysis has been performed with Illumina Miniseq and MinION (Oxford Nanopore Technologies, ONT). Illumina data were analysed with Ridom SeqSphere+. Long-reads sequences were assembled using flye and antimicrobial resistance genes were identified using ABRicate. The blaVIM_regions, that comprehend the blaVIM1 gene, the 1000 bp upstream and the 1000 bp downstream, were aligned and clustered.

Using Illumina analysis, the 27 strains were divided as follow: *E. hormaechei* (20), *P. aeruginosa* (2), *C. freundii* (2), *E. raggenkampii* (1), *P. tohonis* (1), *K. aerogenes* (1) and 23 out of 27 samples harboured blaVIM-1. To gain further insights into the blaVIM region, long-read sequencing with Nanopore was performed on the first 12 isolates leading to the construction of a blaVIM region cluster tree. The analysis revealed the presence of 2 main groups, one including 8 *E. hormaechei* (1 environmental strain and 7 clinical) and one including 2 environmental strains of C. freundii.

The comparison between the coreSNPs tree and the blaVIM_region tree of *E. hormaechei* confirmed the agreement between the two analyses with an overall overlap of the two trees structures. Furthermore, the long-reads sequencing allowed the identification of a common resistance region between the plasmids linked to different species. This common resistance region is characterized by the presence of the blaVIM1 gene and of aac(6')-Ib-G (associated to resistance to aminoglycoside), flanked by restricted sites.

The performed analyses suggest that an intraspecies transmission of the blaVIM region occurred between environmental and clinical strains of *E. hormaechei*; our data support the hypothesis that a recombination mechanism facilitates the intraspecies resistance gene acquisition. Our work demonstrated the importance of long-read sequencing for detecting horizontal gene transmission of resistance determinants and a full plasmid characterization.

P04

Dynamic gastrointestinal tract colonization and fatal bloodstream infection with ESBL-producing-*Klebsiella pneumoniae* ST307 strains in a leukemia patient

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Klebsiella pneumoniae is an opportunistic pathogen that can colonize the gastrointestinal tract (GIT) of humans. Patients with hematological malignancies have their immune system, epithelial barriers, and microbiota impaired, offering a favorable environment for the establishment of bloodstream infections (BSI). When *K. pneumoniae* carry genetic determinants of antimicrobial resistance, such as the high-risk multidrug-resistant *K. pneumoniae* ST307 clone, the resulting infections can be fatal, especially when developed in the hospital setting and treated inadequately. We investigated *K. pneumoniae* strains isolated from GIT colonization (strains Kp_FZcol-1, Kp_FZcol-2 and Kp_FZcro-1) and from a subsequent fatal bloodstream infection (strain Kp_HM-1) in a leukemia patient. Three different genotypes were observed colonizing the patient at the same time, being Kp_FZcro-1 indistinguishable from the infection strain Kp_HM-1 genotype. All strains belong to ST307, carry a transferable IncF plasmid containing the $bla_{\text{CTX-M-15}}$ gene (pKPN3-307 TypeA-like plasmid), have the same resistome and virulome, and are phenotypically multidrug-resistant. Phylogenetic analysis demonstrated that Kp_HM-1 is more closely related to Kp_FZcro-1 than the other colonizing strains. We obtained the circular sequence of a single 246,730 bp plasmid called pKp_HM-1 from the hybrid assembly of Kp_HM-1 genome. When comparing pKp_HM-1 with the other strains genomes, we observed an eighty one percent coverage for Kp_FZcol-2. The Kp_FZcol-2 partial sequences lost were in the putative virulence genes of the glycogen synthesis cluster, *lac* operon, Fec-like iron (III) dicitrate transport system, and glutathione ABC-transport system. To understand more about the plasmid adaptation shown by Kp_FZcol-2, we searched for public genomes of *K. pneumoniae* that had a similar coverage as the Kp_FZcol-2 genome to our reference plasmid pKp_HM-1 and found seven genome sequences with a similar loss. These public genomes were from strains isolated from urine, blood, and diseased humans from different geographic origins, demonstrating that this adaptation of the pKPN3-307 TypeA showed by Kp_FZcol-2 is occurring worldwide and from different sources. We don't know how crucial these putative virulence genes found in the pKPN3-307 TypeA plasmid are for the maintenance and dissemination of *K. pneumoniae* ST307, but we found here that strains lacking a few of these genes seem to still be able to colonize and infect humans. Therefore, we demonstrated that different *K. pneumoniae* ST307 from a common ancestor can coexist in the GIT of humans as well as carry a different variation of the pKPN3-307 TypeA plasmid. We were also able to identify that the infecting strain Kp_HM-1 share a common ancestor with Kp_FZcro-1, suggesting the translocation of bacteria from the GIT to the bloodstream of the patient causing a fatal infection. We strongly indicate the urgency of more studies exploring this dynamic to avoid unfavorable clinical outcomes for immunocompromised patients, as well as the investigation on pKPN3-307 plasmids and their ability to contribute to *K. pneumoniae* ST307 adaptation in the human host and worldwide dissemination.

P05

Characterisation of Mobile Genetic Elements Driving the Global Dissemination of Key Antimicrobial Resistance Genes

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Antimicrobial resistance spreads rapidly and often uncontrollably, due to microbes' capacity to share genes between strains and species. As a result, existing antimicrobial drugs are becoming increasingly ineffective, a crisis that threatens to take 10 million lives per year by 2050 [1]. Dissemination of resistance is primarily driven by mobilisation of existing antibiotic resistance genes (ARGs) to conjugative elements by mobile genetic elements (MGEs). Accurate characterisation of associations between ARGs and MGEs and likely gene dissemination pathways would therefore be invaluable in designing preventative or control measures discouraging the spread of resistance. Here, methods are being developed to fully characterise complex MGE structures responsible for the dissemination of key ARGs. The approaches taken may allow novel structures and associations to be identified and will be applicable to a range of ARG types, with future applications in surveillance and infection control.

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P06

Title: Integron structure and plasmid diversity in clinical VIM-1 carbapenemase producing *Enterobacter hormaechei* isolates from Germany

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Background: The increasing detection of resistance against carbapenems in Gram-negative bacteria is an imminent public health threat. During the last six years, VIM-1 has become the second most identified carbapenemase with an increasing overall abundance and percentage among Enterobacterales isolates as reported by the German National Reference Centre. We analyzed a collection VIM-1 producing clinical isolates collected between 2008 and 2021 of the species *Enterobacter hormaechei* to investigate the dissemination and genetic organisation of *bla*$_{\text{VIM-1}}$ integrons and their plasmid localisation.

Methods: A total of 105 *E. hormaechei* isolates were collected from different German hospitals and subjected to Illumina whole-genome sequencing. In addition, selected isolates were sequenced using nanopore long read technology to reconstruct *bla*$_{\text{VIM-1}}$ integron structures. Genomes were assembled using unicycler and further analysed using an *ad-hoc* core genome multilocus sequence typing (cgMLST) scheme. Plasmid and chromosomal scaffolds were binned and categorized using the software MOB-suite. Resistance genes were identified using ResFinder. Plasmids containing the *bla*$_{\text{VIM-1}}$ integron were further studied by MOB-typing and determination of the Inc group. The reconstructed *bla*$_{\text{VIM-1}}$ plasmids were compared to reference plasmids and further analyzed using average nucleotide identities.

Results: Among the 105 *bla*$_{\text{VIM-1}}$ positive *E. hormaechei* isolates, we identified several potential outbreak clusters using our *ad-hoc* cgMLST scheme. Within some clusters, isolates collected from the same hospital (≤ 7 months) were closely related although their *bla*$_{\text{VIM-1}}$ elements differed in terms of localization on different plasmids and integron structure. This suggests a potential independent acquisition event. Contrary to these highly variable outbreak clusters, other isolates maintained identical cgMLST types, conserved integron structure as well as plasmid localizations of *bla*$_{\text{VIM-1}}$ over years.

Conclusions: Our dataset, while geographically limited to Germany and isolates from the years 2008 to 2021, outlines the impressive diversity among *bla*$_{\text{VIM-1}}$ integrons and plasmids in clinical *E. hormaechei* isolates. Whereas some *E. hormaechei* strains were

remarkably stable in plasmid content over years, others showed different *bla*$_{\text{VIM-1}}$ integron structures and plasmid localization, even within potential *E. hormaechei* outbreak clusters of closely related isolates.

P07

Expanding the arsenal of fertility inhibition factors to fight against the antimicrobial resistance dissemination

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Plasmids play an important role in acquiring and spreading a wide range of antimicrobial resistance genes, posing a significant threat to Global Health [1]. It is therefore desirable to disrupt their mobilization without exerting a positive selective pressure on resistant strains [2]. In nature, plasmids establish interactions that either facilitate or hamper each other's transfer [3]. Fertility inhibition exemplifies one of these interactions, as it suppresses the mobilization of other non-kin co-resident plasmids. Here, we revisit fertility inhibition factors, previously described as isolated examples in a few plasmids, and offer a broader view of their distribution in plasmid taxonomic units and chromosomes. Besides, we examine the fertility inhibition activity of novel factors identified by our phylogenetic approach against a battery of conjugative and mobilizable plasmids. Finally, we search the gene(s) responsible for the fertility inhibition phenotype exhibited by R6K. With this work we increase the arsenal of weapons with which we could target the antimicrobial resistance dissemination.

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P08

Exploration of network structure of plasmids and application to detection of potential transmissions, using Roundhound

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Detecting plasmid transmission presents several unique challenges, among them are the highly flexible structures of genomes, the presence of a small core and few Single Nucleotide Polymorphisms (SNPs), the absence of a sensible measure of genetic distance between plasmids, and the inability to assemble reliably with short reads. While existing tools have provided partial solutions to these issues, they come with their own set of limitations, such as reliance on long-read sequencing, detection of a small subset of plasmids, inability to handle plasmid rearrangements. Moreover, there is currently no method that examines the variants (SNPs, indels) between plasmids.

To address these challenges, we introduce Roundhound, a new tool capable of improving upon the current methodology. Notably, Roundhound is able to identify plasmid transmission with short reads by leveraging on a broad plasmid reference database [1]. It can then be used to highlight isolates for posterior nanopore sequencing. Furthermore, Roundhound provides a higher level of resolution by also quantifying SNPs and insertion/deletion mutations (indels) using Pandora [2].

The functioning of Roundhound can be divided into three primary steps. The first step involves building the plasmid database and network. The database comprises a gene-presence matrix, gene graphs for mapping, a k-mer index, variants information, plasmid clusters, and plasmid distance calculations based on the Double-Cut-and-Join (DCJ) [3] measure.

In the second step, sample reads are queried against this database. Here, Pandora is used to map sample reads against the gene graphs, producing a gene presence matrix and genotyping information on each gene.

The third step involves the inference of plasmid transmission across samples. This is accomplished by identifying plasmids based on gene content, and detecting plasmid transmission through the overlap of plasmid clusters.

Benchmark tests conducted on a dataset with 609 samples [4] have demonstrated the effectiveness of this approach. The results show a recall of 95.6% and a precision of 97.7%, indicating that Roundhound significantly enhances the detection and understanding of plasmid transmission.

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P09

Efficiency of CRISPR-plasmid based resensitivation of AMR bacteria

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The spread of antibiotic resistance among pathogens poses a significant threat to human health. Traditional approaches to combating antibiotic resistance through the development of new antibiotics have slowed in recent years; however, the precision of the revolutionary CRISPR-Cas9 system offers a promising new approach to combating antibiotic resistance. By introducing CRISPR-possessing plasmids that specifically target the antibiotic resistance genes on the resistant ABR plasmids, the susceptibility of the bacteria to antibiotics gets restored and in addition the uptake of the resistance gene is prevented.

However, genetic variations within bacterial populations can hinder the effectiveness of CRISPR-mediated cleavage. Using mathematical modeling and simulations, we investigate the interplay between CRISPR-based plasmid cleavage and genetic variations. We compute the success probabilities of resensitizing a bacterial population with standing genetic variations that impede CRISPR-mediated cleavage of resistance plasmids. Our analysis predicts the success rate of resensitizing treatments and how it depends on the copy number of the plasmid, the compatibility of the CRISPR plasmid and the ABR plasmid, the CRISPR type, the effect of escape mutations, and the number of spacers in the CRISPR array. We find that the usage of incompatible and fast-replicating CRISPR plasmids substantially enhances the efficacy of the treatment. In addition, we investigate how utilizing multiple spacers targeting critical regions of resistance plasmids increases the success probability.

Our predictions emphasize the significance of taking these factors into account during the development and design of CRISPR possessing plasmids for targeting resistance plasmids. These results are a first step towards the design of novel strategies based on plasmid-delivered CRISPR systems to combat antibiotic-resistant strains and hinder the spread of antibiotic resistance without disrupting the microbiome.

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P10

Conjugative-killer plasmids, a novel antimicrobial alternative

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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.

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P11

Multidrug-resistant IncHI2 plasmid in bloodstream infection associated *Salmonella* Typhimurium in Africa

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Sequence type ST313 of *Salmonella* Typhimurium is a major cause of human bloodstream infections in sub-Sahara Africa. In 2022, an HIV-positive man, who travelled from Kinshasa (Democratic Republic of Congo, DRC) to Liverpool (UK), was hospitalised with a *Salmonella* Typhimurium bloodstream infection. The pathogen was isolated from both stool and blood samples, and complete genomes were generated using Nanopore, PacBio and Illumina technologies.

Comparative genomics showed that the chromosome of the stool isolate (designated JH4684) was closely related to the ST313 Lineage 2.0 reference strain D23580 with 51 SNP differences. D23580 carries a pBT1 plasmid, which was previously found to carry essential genes for bacterial survival *in vitro*. However, pBT1 was absent from JH4684. Although the virulence plasmid pSLT was present in both D23580 and JH4684, the $pSLT^{D23580}$ multidrug resistance (MDR) gene cassette was absent from $pSLT^{JH4684}$. JH4684 carried an IncHI2 plasmid that encoded resistance to multiple antibiotics, including Ampicillin, Chloramphenicol, Gentamicin, Trimethoprim, Sulphonamide, Ciprofloxacin and Ceftriaxone. The isolate was susceptible to Azithromycin. A similar plasmid, pSTm-ST313-II.1, was reported in ST313 L2.1 strains from DRC and had a similar antibiotic resistance profile with JH4684 [1].

A large-scale comparative genomic analysis with $pIncHI2^{JH4684}$ identified closely-related IncHI2 MDR plasmids in a variety of sub-lineages of *S.* Typhimurium ST313 and in different Enterobacteriaceae species (visualisation available in Microreact https://microreact.org/project/beTkG2SptznruioeWJr2P5-plsdbkinshasa).

Overall, we have identified a novel bloodstream-associated *S.* Typhimurium ST313 L2.0 strain that is closely related to D23580 but has distinct genomic features. The strain carries an IncHI2 plasmid that was first reported in ST313 L2.1. A variant of the pSTm-ST313-II.1 plasmid has now been acquired by a diverse range of bacterial pathogens in Africa and elsewhere.

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P12

Plasmid versatility in *Cyanobacteria*

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Plasmids are key components in the dissemination of antibiotic resistance amid bacteria. Amongst them, conjugative plasmids are of special interest since potentially they can drive horizontal gene transfer even between distantly related bacteria. Nevertheless, the study of the bacterial plasmidome has been generally restricted to that of the order *Enterobacterales*. While it supposes the most clinically relevant group of bacteria, this has left plasmids that could suppose potential sources of antibiotics resistances in other taxa uncharacterised. This work globally explores the plasmidome of the phylum *Cyanobacteria.* Using bioinformatic tools, we evaluate the cyanobacterial plasmid diversity, host range, replication systems, conjugative transfer potential, protein function enrichment and specificities by cyanobacterial order, and the recent intra-phylum horizontal gene transfer events between plasmids and chromosomes.

P13

Abstract for EMBO Workshop: Plasmids as vehicles of AMR spread

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Many ecologically-important traits are transmitted between bacteria by horizontal gene transfer (HGT), driven by the activity of mobile genetic elements (MGEs). MGEs are modular and routinely interact with one another. This modularity of MGEs may help microbial communities in the face of a changing environment, increasing resilience to stressors by accelerating the spread of adaptive traits between MGEs and across community members. Using computer modelling and a laboratory microcosm system, we investigated how different plasmid 'vehicles' affected the spread of a chromosomal, transposon-borne resistance gene. We found that resistance gene mobilisation varied across a panel of plasmids in a manner largely independent of conjugation rate, suggesting that other plasmid features, such as gene content, 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. The results showed seemingly increased variability in the level of resistance trait maintenance in the final population when the resistance gene was transposable, and on average, resistance traits were maintained at higher levels when non-transposable. Resistance traits were maintained at higher levels under selection compared to without selection. Using computer modelling to mimic the experiment, we found concordant observations of increased variability in the final population with transposable traits compared to non-transposable traits. Varying the strength of selection pressure, showed a seemingly higher probability of transposon dominance at mid-levels of selection. Understanding the nested hierarchies of mobile genetic elements and consequent gene exchange has important application in predicting the evolution of traits like antimicrobial resistance in microbial communities.

P14

Plasmid sharing between cephalosporin-resistant commensal bacteria and enteropathogens in Vietnamese community

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Plasmids in human gut microbiome are the major reservoirs contributing to the global emergence and spread of antibiotic resistance (AMR) genes and bacteria. Frequent horizontal gene transfer (HGT) of plasmids are considered to drive a complex dissemination of AMR across different members of *Enterobacteriaceae*; however, the host reservoirs and transmission dynamics are not completely understood [1, 2]. In Vietnam, considerable efforts have been made to understand the spread of extended spectrum beta lactamase (ESBL)-encoding genes under One Health approach. However, these efforts generally lacked a thorough investigation of the broader ESBL plasmid transmission patterns between ecological niches [3, 4]. In this study, we hypothesised that human gut microbiome constitutes a major reservoir for the ESBL plasmids acquired by the gut-associated pathogens. To test our hypothesis, we first isolated and characterised the genetic structure of cephalosporin-resistant conjugative plasmids from human gut microbiome. We then investigated the extent of sharing between major plasmid groups in human gut microbiome with putative plasmid sequences obtained from human disease-associated pathogens (*Shigella sonnei*, non-typhoidal *Salmonella*, extra-intestinal pathogenic *Escherichia coli* ST131) and animal gut microbiome. These bacterial isolates were all collected between 2010 and 2018 within Vietnam. IncI1, IncB/O and IncF are found to be the most common plasmid groups, carrying mainly $bla_{\text{CTX-M14}}$, $bla_{\text{CTX-M15}}$, $bla_{\text{CTX-M27}}$ and $bla_{\text{CTX-M55}}$, in both cephalosporin-resistant human-derived commensal bacteria and pathogens in Vietnam. Plasmid phylogeny and network analysis reveal the commonality of plasmid sharing between human disease-associated pathogens and human gut microbiome, and also animal gut microbiome but to a much lesser extent. We found that IncI1 and IncB/O ESBL-plasmids can move amongst *S. sonnei,* non-typhoidal *Salmonella* and *E. coli,* with different conjugation frequencies. Plasmids found across different bacterial hosts were associated with higher conjugation frequencies, supporting the crucial role of HGT in determining the degree of plasmid spreading. These results highlight the importance of human gut microbiome as the reservoirs for ESBL plasmids in clinically important gut-associated pathogens in Vietnam and should be targeted for future interventions.

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P15

One Health genomic epidemiology of *Escherichia coli* and antimicrobial resistance within the national Malawian poultry supply chain.

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Understanding the complex epidemiology of WHO-priority pathogens such as *E. coli* and their antibiotic resistance patterns relies on holistic One Health approaches. In this study we use a unique strategy, sampling a hierarchical poultry community breeding structure including poultry, farmers, and their environment across the central region of Malawi as designed and implemented by six fellows funded by Fleming Fund. We use 24 antibiotic enriched metagenomes to map the microbial and resistome landscape. Combined with long and short read sequences of 244 phenotyped *E. coli* isolates to examine the molecular characteristics including but not limited to the role of mobile genetic elements in trafficking antimicrobial resistance (AMR) determinants. Our objective here is to evaluate the contribution of 'vertical' transmission of resistance from founder flocks at the apex, down to multipliers and small-scale farmers, compared to 'horizontal' introduction, along the supply chain. By regarding antimicrobial resistance genes (ARGs), their flanking regions and recombination patterns of plasmids to identify potential sharing events between hosts and dynamic AMR. Our preliminary analysis shows enriched metagenomics selecting for low abundance genes to resistance classes of interest; for example, ciprofloxacin resistant genes mediated by IncX plasmids from *E. coli*. Our long read sequence data allows us to better understand and resolve the genetic context of the ARGs to understand their mobility and sharing patterns across the One Health spectrum.

Here, we capture through a combination of structured sampling, long-read sequencing and genome sequencing, the prevalence and transmission of AMR to identify pathways of AMR spread and the potential zoonotic risk of these within a complex environment. I will present for the first time the phenotypic and molecular characteristics of *E. coli* and its domicile microbiome recovered from a 60-year-old community poultry breeding system in Malawi.

P16

Are Type VI Secretion System-encoding plasmids vehicles for antimicrobial resistances?

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The Type VI Secretion System (T6SS) is a multiprotein complex present in Gram-negative bacteria that delivers effectors into both prokaryotic and eukaryotic cells in a contact-dependent manner, playing a crucial role in interbacterial competition and pathogenicity [1,2]. It has been reported that T6SS mediates horizontal gene transfer (HGT) by incorporating DNA released by the prey cells [3,4]. Plasmids are considered the main vehicles for genetic exchange allowing bacterial populations to adapt to changing environments by the dissemination of adaptative genes such as virulence factors, antibiotic resistances, and degradation of xenobiotic compounds [5]. Here, we searched for the presence of T6SS components across the NCBI RefSeq database and assessed the abundance and distribution of T6SSs in plasmids hosted in different bacteria. The diversity and relatedness of these T6SSs were analyzed through a phylogenetic reconstruction, revealing the T6SS^{i} subtype as the most abundant. T6SS^{+} plasmids were assigned to their corresponding plasmid taxonomic unit (PTU) and their potential mobility was evaluated. We explored the simultaneous presence of T6SS and antimicrobial resistance (AMR) genes, finding no association between T6SSs and AMRs in plasmids. Only 28 out of the 533 T6SS-encoding plasmids also encoded AMR genes, which belonged mainly to the beta-lactam (19 genes), aminoglycoside (17), sulphonamide (12), tetracycline (12), phenicol (7), fluoroquinolone (5) and trimethoprim (5) resistance classes. Despite the scarce presence of AMR in T6SS-encoding plasmids (20 plasmids contained 1 AMR gene), 4 of them from *Enterobacterales* were multidrug resistant (MDR), carrying the clinically relevant AMR family gene *bla*$_{CTX\text{-}M\text{-}X}$ that confers resistance to ceftriaxone.

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P17

Emergence of Co-trimoxazole, Nitrofurantoin, and Fosfomycin resistance among bacteria isolated from UTI patients and environmental wastewater

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Antibiotic resistance, a natural phenomenon, is the ability of microorganisms to resist the effect of an antibiotic to which they were once sensitive, which cause around 300 million premature deaths and a loss of up to $100 trillion (£64 trillion) to the global economy by 2050 [1]. Resistance to first-line antibiotics and, more recently, last-line treatments, and even combination drugs could be fatal in case of minor infections. Co-trimoxazole, a combination of two antifolate compounds - sulfamethoxazole and trimethoprim, Nitrofurantoin, and Fosfomycin are effective against Gram-negative and Gram-positive bacteria. These antibiotics are listed as critically important antimicrobials for human medicine in WHO handbook and thus the study of resistance against these antibiotics is of major importance. Horizontal gene transfer (HGT) occurs through transformation, conjugation and transduction is mainly responsible for the widespread of antibiotic resistance genes among bacterial isolates [2]. HGT also decipher the finding of antibiotic resistant bacteria (ARB) and genes (ARG) in different environments and livestock through the transfer from environmental into the clinical pathogens. The transmission of ARGs among different bacterial species is a global human threat. This study aims at determining the prevalence and diversity of co-trimoxazole, nitrofurantoin, and fosfomycin resistant bacteria in clinical and environmental wastewater. Furthermore, this study will further provide in-sights into the antibiotic resistance spread in clinical and environmental samples and how these two systems interlink.

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P18

Ecology & evolution of a clinical plasmid population

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The thousands of clinical samples routinely collected and sequenced for infection control in hospitals generate a fantastic opportunity to study the evolution of plasmids and their bacterial hosts. The generated datasets contain snapshots of multiple evolutionary timescales, from the distribution of plasmids at the population level to instances of short-term evolution using transmission clusters and longitudinal sampling of individual patients.

We investigated the ecology and evolution of plasmids in *E. coli* in a patient population by analyzing all *E. coli* isolates collected over a year in one US healthcare network. This dataset represents more than 2000 samples, for which we have both short-read data and antibiotic resistance phenotype. In this dataset, we identified a cluster of plasmids similar to the IncF plasmid pUTI89 [1] present in more than 30% of all samples (both urine and non-urine). This highly successful cluster carried virulence factors but little to no antibiotic resistance genes and was surprisingly widespread across close to all *E. coli* sequence types.

First, we use the 637 samples containing coverage for at least 90% of the 120kb pUTI89 genome as a case study for plasmid evolution. Focusing on the conserved backbone, we are investigating the nucleotide diversity along the plasmid genome and especially the conjugation machinery to identify potential signals of diversification in response to plasmid-dependent phage predation, as well comparing the rate of evolution of plasmids in comparison to their bacterial host.

We then investigate the ecology of all IncF plasmids in the population and their impact on antibiotic resistance. F plasmids are known to be important carriers of antimicrobial resistance genes in Enterobacteriaceae [2] and more than 80% of all samples in our dataset carried an IncF plasmid. We found that pUTI89 presence was associated with reduced number of resistance genes and lower resistance levels when compared to samples carrying other IncF plasmids. As plasmids the same incompatibility type struggles to coexist within the same cell and exclusion mechanisms found on IncF plasmids hinder co-infection [3], we are investigating the hypothesis that the presence of the sensitive pUTI89 plasmid could limit the spread of resistant genes carried by other F plasmids in the population. The prevalence and dynamics of resistance genes in a bacteria population may therefore be impacted not only by the selection pressure generated by antibiotic use but also by the interactions between their plasmid vectors, even the ones that do not carry resistance.

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P19

Linking genetic diversity of the IncF transfer region to plasmid spread

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IncF plasmids are important vectors of horizontal gene transfer due to their conjugative potential and widespread prevalence in clinically important Enterobacteriaceae (e.g *E. coli*, *Salmonella* and *Klebsiella*) [1, 2, 3]. The genes responsible for mating pair formation and mobilization in the IncF plasmid have diverse regulatory schemes and vary in their conservation and co-occurrence [2, 4]. However, how this diversity is linked to plasmid spread in a specific ecological niche is not well described. Using a large, curated collection of 4201 public *E. coli* genomes associated with food animals (swine, poultry and cattle) [5], we aim to link the genetic diversity, co-occurrence and co-variation patterns of genes responsible for conjugation of IncF plasmids to population dynamics of plasmid transmission. We also aim to quantify the degree of selection acting on these genes (using dN/dS ratios) and assess whether genes known to interact in the conjugative apparatus co-vary in a similar fashion.

In a preliminary analysis of a subset of 87 assemblies from *E. coli* isolated from pigs in the UK, we find putative IncF like transfer genes in 64% of genomes, with 50% of the genomes having more than two-thirds of the genes known to be part of the F plasmid transfer region. Interestingly, the entry exclusion gene *traS*, previously described to be essential for the stability of conjugative plasmids [6], is present only in a quarter of the putatively mobile IncF plasmids. The rarity of *traS* in our dataset points to interesting implications regarding plasmid competition and rates of successful conjugation among hosts with similar plasmids.

We are extending this initial characterization to the rest of the genomes in our data set. Considering the clinical importance of IncF plasmids in antibiotic resistance transfer [7], linking genetic diversity to plasmid transmission can help estimate the extent to which conjugative horizontal gene transfer shapes the spread of antibiotic resistance genes in natural populations.

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P20

Sinks as hotspots of plasmid evolution and dispersal during a carbapenemase-producing *Enterobacteriaceae* outbreak at a hospital

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Carbapenemase-producing *Enterobacteriaceae* (CPE) threaten our health system by reducing treatment options during infection. Plasmids carry many of the resistance loci and thus proper infection control relies on preventing the spread of these genetic elements on top of the bacteria that carry them. Here, we show that sinks in hospital rooms serve as reservoirs for CPE during an outbreak at Sheba Medical Center from 2017-2019. We routinely, longitudinally sampled both sinks and patients and further characterized isolates using Pulsed-Field Gel Electrophoresis and identified carbapenemase genes using Xpert Carba-R PCR. We found different species of *Enterobacteriaceae* that carried different resistance loci (VIM, OXA-48, NDM-1, KPC) sustained the outbreak. Both populations of cells and resistant loci persisted in sinks over weeks with many patient isolates mirroring those from the sink in their treatment room. We also observed switching between which resistance loci were carried by the same species. To look at this more closely, we used hybrid assembly to fully resolve the genomes of 4 isolates, pairs of sink and patient isolates from two different rooms [1]. One of the paired samples, two *Klebsiella pneumoniae* isolates, shared a plasmid conferring carbapenem resistance, based on genetic similarity, while differing in the overall genomic content, based on features of the other carried plasmids. This serves as preliminary evidence that plasmid mobility itself can drive nosocomial infection, in addition to dispersal of antibiotic resistant cells.

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P21

PREVALENCE OF ANTIBIOTIC RESISTANT BACTERIA AND THEIR RESISTANT PATTERN AMONG URINARY TRACT INFECTION SUSPECTED PATIENTS VISITING TERTIARY CARE HOSPITAL OF NEPAL.

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ABSTRACT

Urinary Tract infection (UTI) caused by antimicrobial resistant (AMR) bacteria is a significant problem in all patients leading to high morbidity, poor quality of life and a limited life expectancy [1]. Thus, this study aims to identify uropathogens and determine their antibiotic susceptibility in UTI suspected patients. For this mid-stream urine sample from the UTI suspected patients was cultured and bacteria was isolated and identified following the series of biochemical test method [2]. Antibiotic Susceptibility testing was performed according to Kirby Bauer disc diffusion method [3]. Thus obtained result was analyzed using SPSS_20 and WHONET2020. Ethical approval from concerned authority was taken before proceeding (NHRC Reg.no. 169/2020). This was a funded research from University Grant commission, Nepal (SRDI-75/76-S&T-11) but due to limited budget molecular analysis could not be performed. In this study a total of 184 urine sample was found to be culture positive out of 1784 suspected cases within the time frame of June 2020 to March 2021. Different species of bacteria was isolated and identified, among which *E. coli* was predominant followed by *Klebsiella spp., Proteus spp., Staphylococcus spp., Pseudomonas spp., Morganella spp. and Acinetobacter spp.* Antibiotic resistant pattern was observed highest among *Klebsiella spp.* against the major antimicrobials. Among the 182 isolates higher resistance was observed towards Cefoxitin (70.3%) followed by Ceftazidime (70.2%), Nalidixic acid (67.6%), Cefixime (62.5%), Amoxicillin clav (59.3%), Gentamicin (57.1%), Polymixin B (54.1%), Ciprofloxacin (53.4%), Norfloxacin (50.5%), Amoxicillin (50%) and Colistin (50%). A total of 29 multi drug resistance (MDR) isolates were detected and some of their resistance profile showed that they could be possible Extreme Drug Resistant (XDR). Additionally, WHONET2020 software predicted 13 (7.06%) isolates that were resistant to Carbapenem class of antibiotics as high priority isolates. To summarize, AMR among urine isolates seems to be rising so situation analysis is the need of the hour. Further, not just phenotypic prevalence but molecular analysis of AMR bacteria is highly required. This molecular analysis can help understand the trend and dissemination pattern of such isolates at gene level and their comparison with the global prevalence will help understand the global trend as well. This will ultimately play a vital role in intervention of AMR evolution and dissemination.

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P22

Full Title (13/50 words):

In silico investigation of the plasmid composition of over 400 Enterobacteriaceae strains held by the National Collection of Type Cultures.

Authors

Jake David Turnbull, Sarah Alexander, Jo Dicks

Abstract (224/250 words):

The National Collection of Type Cultures (NCTC) is composed of 6,000 medically relevant bacterial strains maintained by the UK Health Security Agency (UKHSA). The genomes of approximately 3,000 NCTC strains have been long-read sequenced, as a part of the NCTC_3000 project. This wealth of genomic data provides an opportunity to refine what is known to date about the plasmid content of the sequenced NCTC bacterial isolates.

Here, we apply a series of bioinformatic tools and approaches to approximately 400 assemblies derived from strains of *Escherichia coli* and *Klebsiella pneumoniae*. We identify contigs of plasmid provenance and type contigs deemed to be of plasmid provenance based on *rep* and *mob* loci and determine their AMR gene content. Genome assembly optimization (e.g. re-assembly using different assemblers and the addition of short read data) in a select strain set was also performed to investigate whether the current genomic assemblies adequately reflect the true plasmid content of the strains in question.

The resultant analysis details the most comprehensive description of the plasmid population of NCTC *Escherichia coli* and *Klebsiella pneumoniae* strains carried out to date, revealing the NCTC strain set to be a rich source of diverse plasmids. This dataset, containing both previously described and putatively novel structures, has been contextualised using the available meta-data associated with the strains, and allows commentary on plasmid evolution across human history.

P23

Species-wide evolutionary dynamics and virulence gene flow across *Escherichia coli*

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Enterotoxigenic *Escherichia coli* (ETEC) colonisation factors have typically been associated with specific *E. coli* lineages. In this study, we have analysed 79,034 *E. coli* genomes using PopPUNK, grouping them into 251 lineages. Our research reveals that the relationship between colonisation factors, toxins, and lineages is far more complex than previously assumed. About half of the genomes exhibited both toxins and colonisation factors, while the rest either had colonisation factors but no toxins, or toxins without known colonisation factors. This indicates a nuanced interplay between colonisation factors and toxin presence.

Contrary to previous research which narrowly focused on human-isolated ETEC genomes, our study investigates a broad spectrum of *E. coli* lineages. We identified hybrid colonisation factors in 937 genomes, which are currently classified into eight variants. These have been detected in a range of pathogenic *E. coli* strains from diverse geographical locations, revealing a complexity that was previously underestimated.

Further, we explored the diverse backbones containing ETEC virulence genes using Nanopore long-read sequencing. Our preliminary data contradict the long-held belief that colonisation factors are exclusively located on plasmids, revealing that a subset also resides on the chromosome. This key insight will help us better understand the mobility of these factors within the *E. coli* phylogeny.

In conclusion, our research sheds light on the complexity of colonisation factor distribution in *E. coli*, hybrid pathotypes, and the plasmid/chromosome location of colonisation factors. It underscores the need for further investigation into the evolution of pathogenic *E. coli*, which has implications for managing *E. coli*-related infections in both humans and animals.

P24

Laboratory evolution of antimicrobial resistance through horizontal gene transfer in bacterial communities

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Antibiotic resistance is frequently acquired through horizontal gene transfer (HGT) rather than through mutations inherited vertically. However, the majority of laboratory evolution experiments have focused on single-strain bacterial populations, while communities that are more prevalent in nature have received little attention. To better study the dynamics of horizontal gene transfer of antimicrobial resistance we are developing an *in vitro* system. In particular, we are using synthetic *E. coli* communities grown in a culturing device (Chi.Bio), which is capable of tracking a continuous culture in real time and importantly how its composition changes when a selective pressure is introduced. This system can therefore detect gene transfer and community dynamics during adaptation to antimicrobials. I will show our preliminary results on establishing this system and a detailed research plan that involves the use of a synthetic community built from fluorescently-tagged auxotrophic *E. coli* strains.

P25

A continuously evolving DNA barcode for lineage tracking

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DNA barcodes use static sequences to identify cellular lineages. Two key limitations of barcodes are that they lose utility after a selective sweep and that they are blind to within-lineage differences. In contrast, an evolving barcode that progressively changes its sequence over time could be used to reconstruct a precise lineage history for all cells. During experimental evolution, pairing barcodes with WGS would allow high-resolution measurement of evolution-in-action. CRISPR-guided systems have been proposed and previously used for this purpose. However, previous evolving barcodes face the problem of limited mutability— a mutation destroying the PAM site of a barcode renders it static. Further, the mutation rate of the barcode would ideally create a diversity large enough to allow lineage tracking in cells undergoing rapid selection.

We achieve this with a construct that fuses a low-fidelity DNA polymerase to a CRISPR-guided nickase (EvolvR[1]) which targets its own gRNA locus (homing guide RNA[2]). We show that selfEvolvR has *in vitro* and *in vivo* activity in *E. coli* when expressed over 100 generations, and intend to repeat this quantification with chromosomally-integrated selfEvolvR. Currently, we are constructing a library of mutant DNA polymerases in order to maximize barcode mutation rate.

Once optimized, we propose that selfEvolvR has applications reconstructing phylogenies during long term evolution experiments, and particularly those that involve disentangling the vertical and horizontal transmission of plasmids in a population where only some cells have a plasmid. At a population level, deleterious fitness effects of a plasmid can be masked by increased horizontal transmission; having chromosomally barcoded lineage would allow for direct observation of this effect. In all, we propose that the selfEvolvR system has a potential for a variety of applications in microbial evolution research.

[1] Halperin, S.O., Tou, C.J., Wong, E.B. *et al.* Nature **560**, 248-252 (2018).
[2] Kalhor, R., Mali, P., Church, G., Nat. Methods. **14**, 195–200 (2017).

P26

Diversity and spread of plasmids among *Klebsiella pneumoniae* in Norway

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Klebsiella pneumoniae is a global opportunistic pathogen and frequent cause of a wide array of nosocomial and community-acquired infections. *K. pneumoniae* can colonize humans and animals and may be found in both terrestrial and marine environments. Strains that cause nosocomial infections often have acquired antimicrobial resistance (AMR) genes, the vast majority of which are plasmid-borne. Through recombination events and host range expansion, plasmids exacerbate the accumulation and circulation of AMR genes amongst *K. pneumoniae* via horizontal gene transfer (HGT). There remain knowledge gaps regarding the determinants of persistence and transmission of AMR genes in *K. pneumoniae* within and across sequence types (STs), as well as ecological niches. Understanding the fundamental mechanisms that govern successful plasmid, and therefore AMR, transmission is critical to develop effective AMR prevention strategies. Here, we investigate plasmid diversity and factors that influence plasmid transmission across STs and niches in *K. pneumoniae*, and therefore drive the spread of AMR.

We short-read whole-genome sequenced 3255 *K. pneumoniae* isolates collected in Norway between 2001 and 2020 from three ecological niches: human (n=2656), animal (n=500), and marine (n=99). Of those, a representative 477 (15%) isolates were also long-read sequenced to produce high-quality hybrid closed genomes. The hybrid-assembled plasmid sequences will be used to assess the remainder of the collection. Genotyping was performed using Kleborate v2.0.0, Abricate v1.0.1, PlasmidFinder database v2021-11-29, and MOB-suite v3.1.0.

Of the 477 hybrid-assembled genomes, 386 (81%) contained plasmids, and 151 (32%) isolates carried >1 plasmid. Altogether we found 577 plasmids, of which 115 (20%) carried AMR genes. Plasmids were characterized in terms of replicon and predicted mobility types to evaluate diversity, and to explore associations of plasmid types with host niches and STs. Further work will examine these associations, as well as co-localization of plasmids and AMR genes to identify key factors that may influence plasmid transmission and stability.

P27

Horizontal Plasmid Transfer Drives Antibiotic Resistance in Intensive Aquaculture Farms

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Genetic transfer, including horizontal plasmid transfer (HPT), drives bacterial evolution by mediating the sharing of adaptive traits, such as antibiotic resistance (AR), between bacteria. However, mechanisms of HPT in natural environments with multiple bacterial populations and diverse plasmids remain unclear. We integrated culture-based and culture-independent-techniques to assess the role of HPT in the emergence and spread of antibiotic-resistant genes (ARGs) in intensive frog farms, a typical yet understudied hotspot of AR. Metagenomic analysis of sediments, combined with high throughput antibiotic susceptibility testing of bacterial isolates revealed *Escherichia coli* and *Edwardsiella tarda* as the core antibiotic resistant bacteria (ARB) in frog farms. Completed genomes of 95 multidrug-resistant (MDR) bacterial isolates (mainly *E. coli*, *E. tarda*, *Citrobacter*, and *Klebsiella*) were constructed. A total of 250 large plasmids (average size >110 kb), that harbored diverse ARGs flanked by mobile genetic elements (MGEs), were identified from the 95 MDR isolates. Many of the plasmids (57%) were conjugative and some (20%) were multi-replicons. Most of the plasmids (62 %) fell into 32 distinct groups that tracked ARG dissemination *via* inter- and intra-species HPT. The AR phenotype strongly correlated with plasmid-born ARGs instead of chromosome-borne ARGs. Multiple plasmids in various combinations (2-3 per host), were also shown to preferentially co-habit to achieve the desirable AR phenotypes for adaptation. Same mobile ARGs (contigs with both ARGs and MGEs) were found in different locations in the same plasmids, and in different plasmids from the same bacterial hosts. Moreover, similar mobile ARGs cassettes existed in different plasmids in inter-/intra- species, indicating that ARGs were horizontally transferred among plasmids. Blasting public datasets showed that plasmids highly identical to those from the frog farms were present in other environmental niches globally. These findings demonstrate the importance of understanding the mechanisms of plasmid and other MGEs mediated horizontal gene transfer for developing strategies to combat the spread of antibiotic resistance.