334A - Functional metagenomics for the investigation of ancient microbial antibiotic resistance in permafrost

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Permafrost, or permanently frozen soil, is found in polar regions and underlies ~20% of the Earth's surface. Despite subzero temperatures, permafrost hosts a diversity of microbial life. Permafrost serves as a substrate in which to study survival strategies in extreme cryoenvironments. To understand microbial community survival, we focused on a particularly important aspect of community function--antibiotic resistance. Antibiotic resistance is ancient and occurs naturally among soil dwelling microbes. Antibiotic resistance genes are involved in community signaling, environmental sensing and play a role in competition and defense interactions. To identify antibiotic resistance genes in bacterial communities that have never been exposed to modern synthetic antibiotics and to determine their importance as a survival strategy, we employed functional metagenomics. Using this approach, we extracted DNA directly from permafrost frozen for 19,000 to 33,000 years before present and cloned it into a plasmid vector. A metagenomics library was thus constructed and expressed in an *E. coli* surrogate host. We screened for antibiotic resistance, pooled resistant colonies, and sequenced the antibiotic resistance genes using high-throughput next-generation sequencing. Results obtained from an analogous permafrost substrate demonstrated a robust metagenomic library size of 90.9 GB with 1 out of 15,000 clones expressing antibiotic resistance. Sequence data confirmed the transfer and identification of antibiotic resistance genes from both gram positive and gram negative microorganisms as well as novel functional resistance genes. These results expand our knowledge of functional resistance genes and reveal specific modes of action diverse microbes must use to survive in extreme environments.

335A - Variation in genotype and phenotype of resistance to antibiotics involved in collateral sensitivity interactions in Escherichia coli

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As antibiotic resistance spreads there is a lot of interest in using rational treatment strategies to slow the evolution of resistance. One strategy is to use combinations of drugs where mutations causing resistance to one drug sensitise the bacterium to one or more other drugs. Thus far work on this pleiotropic effect, termed collateral sensitivity or negative cross resistance, has focused on finding which pairs of drugs have collateral sensitivity interactions. However, little is known about whether the emergence and expression of known collateral sensitivity varies with environment.

To investigate this, we isolated and sequenced independent mutants of a lab strain of *Escherichia coli* with resistance to the minimal inhibitory concentrations of five antibiotics (gentamicin, streptomycin, chloramphenicol, cefuroxime and trimethoprim) in four different lab environments. We then tested mutants, in the

same four environments, for resistance to the antibiotic they were selected against, as well as other antibiotics previously implicated in collateral sensitivity.

This work gives important new information on how selection for antibiotic resistance is affected by environmental conditions for important and interesting antibiotics involved in collateral sensitivity interactions.

336A - Review on the global distribution of plasmid-mediated AmpC beta-lactamases in aquatic systems

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AmpC beta-lactamase genes are one of the most prevalent antibiotic resistance groups worldwide and forms a part of the extended spectrum beta-lactamases (ESBLs). When incorporated in plasmids, they are unregulated and readily disseminated among various bacterial groups. These genes are well documented in clinical settings. However, paucity of knowledge of plasmid-mediated AmpC genes in aquatic environments exists. This review was aimed to bring to light the gap in literature regarding the prevalence of plasmid-mediated AmpC beta-lactamase genes in aquatic systems. A total of 950 literature sources were identified by a predetermined keyword search in 27 databases of which 24 journal articles were selected for full text analysis. Data on plasmid mediated AmpC genes from 16 countries. These were from surface water (12), wastewater (7), sea water (3) and both surface & wastewater (2). The estimated study areas of these articles cover less than 1% of the total surface of the earth. The majority of studies were not specifically aimed for the detection of AmpC genes in order to assess ecosystem health, but rather detected along with other antibiotic resistance genes (ARGs). At this time, no surveillance and environmental limits exist for these genes and due to the lack of research, it is unlikely that such systems will be implemented in the near future. The implications and dynamics of plasmid-mediated AmpC genes in aquatic systems remain unclear and require intense research in order to generate sufficient data that could be used to address global spread of antibiotic resistance.

337A - Associations between reduced susceptibility to antibiotics, disinfectants, and heavy metals in natural and clinical isolates of *escherichia coli*

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Understanding all the factors contributing to the challenge of antibiotic resistance is key in order to find solutions to it. In non-laboratory environments, bacteria often come in contact with disinfectants, such as triclosan and quaternary ammonium compounds, and heavy metals, e.g. zinc and cadmium (used as in-feed growth promoters in agriculture and in batteries, respectively). Evolution of reduced susceptibility to these compounds has been observed for various strains and in diverse conditions, and the underlying mechanisms are in many cases the same as

known for antibiotic resistance (e.g. increased expression of unspecific efflux pumps and degradation enzymes). However, it is not yet clear how a reduction in susceptibility to disinfectants and heavy metals is associated with the evolution of antibiotic resistance in non-laboratory strains. To address this knowledge gap, we will test a well-characterized collection of 94 natural and clinical *E. coli* isolates for their susceptibility to a selection of disinfectants and heavy metals and subsequently map the results against already existing antibiotic resistance profiles of these isolates. Screening of the library of isolate sequences for the genetic basis of detected associations between antibiotic resistance and reduced susceptibility to disinfectants and heavy metals and the creation of knock-out strains will help elucidate the mechanisms shared between resistances to different antimicrobial compounds.

338A - Do agricultural antibiotics cause selection and dissemination of antimicrobial resistance in the bee gut microbiome?

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Prophylactic antibiotic treatment of bee hives inadvertently causes accumulation of mobile antibiotic resistance genes within bee gut microbiomes. Another unexplored route of exposure of bees to antibiotics is through antibiotic treatment of bee-pollinated crops: several countries treat fire blight infections in apple orchards with streptomycin.

We predict that bees will ingest antibiotic residues on flowers, and could act as mobile reservoirs and disseminators of antibiotic resistance genes through horizontal gene transfer within and between their gut microbiomes. Bees socially transfer their gut microbiome, excrete gut bacteria on flowers, have large flight ranges and are commercially transported around countries. Consequently, resistance genes within their microbiomes will have vast ecological connectivity, and could spread to important plant pathogens. We aim to investigate the evolution and horizontal transfer of antibiotic resistance genes within bee microbiomes, and their dissemination to 1) bees of the same and different colonies, 2) different bee species, and 3) to plant pathogens.

In a preliminary study we experimentally exposed bumblebees (*Bombus terrestris*) to therapeutic doses of antibiotics and used qPCR, sequencing and culture-based techniques to compare the resistance profiles of their microbiomes.

A single exposure to streptomycin at 50 µg/ml severely reduced gut bacterial abundance, which did not recover after 5 days. However, the prevalence of mobile resistance gene indicators, Int1 and IS1133, remained unaffected.

This PhD project pioneers research into the role of bees and other pollinators, as disseminators of antimicrobial resistance, with potential to inform policy on antimicrobial use in the treatment of insect-pollinated crops.

Poster Pitch

339A - Dissecting the bistable switch governing the activity of a mobile element in *Pseudomonas*

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Evolution has shaped a myriad of genetic programs allowing bacteria to colonize specific niches and adapt to changing conditions. More surprisingly, among populations of isogenic cells, adaptive strategies have evolved that lead to subpopulations displaying strong phenotypic differences. The case we study here is a bistable program invoked on host cells of the bacterium *Pseudomonas knackmussii* by a mobile genetic element named ICE*clc*. In most cells, ICE*clc* is and remains stably integrated in the bacterial chromosome, but in 3-5% of cells the element becomes active, leading to its excision as a circular molecule that can transfer to other cells by conjugation. Transfer requires the initiation and completion of a differentiation program named transfer competence development, but the mechanisms controlling the bistable switch between non-active and transfer competence have remained unclear. Using targeted mutations, RNA-seq, single-cell fluorescent ICE*clc*-promoter-reporter assays and transfer experiments, we dissected the genetic network responsible for ICE*clc* activation. We further reconstructed the minimal gene set necessary for developing bistability in a *Pseudomonas* strain devoid of ICE*clc* and showed how to control variable outputs. A stochastic mathematical model was built that explains the network structure necessary to generate bistability. This model may be further used to describe different types of bistability in various biological processes.

340A - Unveiling resistome profiles in the sediments of Whalers Bay, Deception Island, through metagenomics

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In the 20th century, Whalers Bay of Deception Island, Marine Antarctic, was known as a port where whalers were established for processing whale oil. At present, the island is one of the most visited by tourists. The extent of anthropogenic impact of human activities is unknown and there are no studies of microbial resistance mechanisms at Whalers Bay. Therefore, the aim of this work was to perform resistance analysis along a biofilm formed on top of sediments at Whalers Bay using metagenomics. Samples were collected by the research team of MycoAntar Project during the summer 2014-15. Total DNA extraction of four samples along a transect

of 27 m was performed in duplicate, followed by sequencing using Illumina HiSeq platform. Bioinformatic analysis was performed for construction of scaffolds and functional annotation with Kegg orthology. Taxonomic annotation revealed *Polaromonas* as the most abundant genus, indicating versatile resistance mechanisms related to changes in abiotic factors. Arsenic, copper, and iron were the most abundant metal resistance classes. For antibiotics, beta-lactam was the most prevalent resistance class, corroborating with previous studies of Antarctic resistome. The acridine class was the most abundant amongst the toxic compound resistances, related to a class of topical antiseptics. Results gathered in this study demonstrated a wide variety of resistance classes to antibiotics and toxic compounds not found in Antarctic, suggesting that a single mechanism used to protect organisms from metals naturally found in the environment may serve to prevent susceptibility to other compounds, such as antibiotics, resulting in adaptive advantage.

341A - Metabolites of actinomycetes isolated from Mexican jungle soils with microbial activity in new molecular targets of human pathogenic bacteria

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Actinomycetes are bacteria with a wide secondary metabolism, which generates molecules with potential antimicrobial activities. In this work, the inhibition of microbial growth and modification of phenotype features related to virulence by supernatants and volatile compounds produced of actinomycetes isolated from jungle soils were assayed. A total of 15 supernatants displayed antimicrobial activity against drug-resistant strains of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae, Acinetobacter baumannii, Escherichia coli*, *Staphylococcus aureus*, *Aeromonas hydrophila* and *Salmonella* Typhi and Typhimurium. Four actinomycete strains produced volatile compounds capable of inhibit the growth and adherence of *K. pneumonia*, *P. aeruginosa* and *A. baumannii*. Meanwhile, other supernatants from actinomycete inhibited the *quorum sensing* of *Serratia marcencens*. Also, five actinomycete supernatants interfered with the iron acquisition of *S.* Typhi and *E. coli* strains. All the bioactive relevant strains were identified by molecular methods as *Streptomyces* spp. The metabolites of the supernatants maintain a potential to disturb the ecological adaptation, colonizing and surviving of pathogen bacteria into the host.

342A - Mobility and persistence of integron-associated antibiotic resistance genes in manure-applied agricultural soil and water

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Amending agricultural soils with manure derived from antibiotic-treated livestock implies the introduction of antibiotic-resistant bacteria into the environment. Furthermore, the capacity of bacterial integrons as accumulators and disseminators of antibiotic resistance genes, in addition to the integron-inducing selective pressures found in livestock guts and manure retention pits, makes manure a potential vector for enhanced resistance gene transfer. We designed a soil column experiment to simulate a 108-day drainage season on artificially drained cropland typical of the Upper Midwestern United States to determine the extent to which integron-harboring bacteria and integron-associated antibiotic resistance genes move throughout the soil and water and persist in the environment post-manure application. Shotgun metagenomes derived from manure-applied topsoil and effluent samples collected after six rainfall events were analyzed to assess the abundance and diversity of integron-integrase genes and integron cassettes. A total of 40 integron cassettes that were not detected in the manure-free control soil or effluent were found to persist in the manure-applied topsoil and in the effluent collected after rain events for at least 108 days post-manure application. Of these cassettes, 23 included genes conferring resistance to antibiotics such as tetracycline, sulfamethazine, aminoglycosides, chloramphenicol, lincosamides, streptomycin, and quaternary ammonium compounds. These results highlight the importance of developing monitoring plans that include assessment of the risk for mobility of antibiotic resistance genes in the environment and the potential of certain environments to encourage the accumulation of mobile antibiotic resistance genes into multi-drug resistant pathogens.

Poster Pitch

343A - Validation of a high-throughput quantitative metagenomic approach to study dynamics of antibiotic resistance genes

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Quantitative PCR (qPCR) alone is not sufficient to observe the spatial and temporal changes in antibiotic resistance gene (ARG) diversity in environmental samples. Previous studies have applied whole-community metagenomic sequencing to monitor changes in relative abundances of ARGs. Although statistical methods have improved to reduce spurious correlation in compositional data such as relative abundance, there are still limitations. The aim of this study was to develop a high-throughput, high-data-magnitude method to deliver gene-centric quantification based on metagenomics to a degree equivalent to qPCR.

We extracted DNA from dairy manure samples and spiked genomic DNA obtained from a marine bacterium not present in manure as an internal standard to establish a relationship between molecules of DNA (i.e., gene counts in sample) and sequenced read counts. This approach enabled quantification in units comparable with qPCR (ARG copies per sample mass, per sample volume, or per 16S rRNA gene copies). We compared abundances to other quantitative and semi-quantitative metagenomics methods. Additionally, we will determine the sensitivity of our

approach and compare the limit of quantification and limit of detection to digital PCR quantification of *sul1, sul2, tetM, tetA, ermT*, and *emrB* genes.

Our quantitative metagenomics method demonstrated that compost harbored the most diversity in resistance genes. ARG families abundant in raw manure samples, such as tetracycline protection proteins and lincosamide inactivation enzymes, were greatly reduced in compost. This gene-centric metagenomic quantification tool will facilitate quantitative studies of microbial communities in environmental systems for any relevant gene groups.

344A - Petroleum contamination and bioaugmentation in bacterial rhizosphere communities from *Avicennia Schaueriana*

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Accidental oil spills are typical sources of urban mangrove pollution that may affect mangrove bacterial communities as well as their mobile genetic elements (MGEs). To evaluate remediation strategies, we followed over the time the effects of a petroleum hydrocarbon degrading consortium (PHDC) inoculated on mangrove tree *Avicennia schaueriana* against artificial petroleum contamination in a phytoremediation greenhouse experiment. Interestingly, despite plant protection due to the inoculation, denaturing gradient gel electrophoresis (DGGE) of the bacterial 16S rRNA gene fragments amplified from the total community DNA indicated that the overall bacterial community composition did not significantly shift. However, while the bacterial community was rather stable, Southern blot hybridization (SBH) analysis revealed pronounced shifts in the abundance of bacteria carrying plasmids, indicating that horizontal gene transfer is a crucial parameter in the development of effective PAH-degrading microbial communities occurring in mangroves. DGGE of naphthalene dioxygenase (*ndo*) genes amplified from cDNA (RNA) indicated the dominance of a specific *ndo* gene in the inoculated petroleum amendment treatment. The results of this study aid in elucidating the response in bacterial community composition to oil contamination in mangrove ecosystems, indicating that a bioaugmentation strategy mediated by highly efficient PDHC can be a useful tool to clean-up the environment. Moreover, the main novelty of this work regarding the PDHC results is that *Comamonas* sp. and *Ochrobactrum* sp. carrying IncP-9 plasmids are reported here for the first time. Thus it seems that under condition with strong selective pressure the host range of IncP-9 plasmids is far broader as previously reported.

345A - Occurrence and dissemination of antibiotic resistant genes under specific micro-eco-environment in sewerage system

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The frequent use of antibiotic in human therapy and animal husbandry has enriched the abundance of antibiotic resistance genes (ARGs) and promoted the development of global antibiotic resistance. The sewerage is the first reservoir of ARGs and might be a hot spot of resistance dissemination, but it is remain poorly understood. This study aimed to investigate the distribution of ARGs and mobile genetic elements (MGEs) in sewers, and tried to explore the character of the ARGs/MGEs transfer and co-occurrence between ARGs/MGEs and antibiotics under specific independent ecosystem. Different antibiotics and ARGs were monitored every other month along the sewers from neighborhood to waster water treatment plant during the whole year, with the method of LC-MS/MS, real-time quantitative polymerase chain reactions and metagenomic sequencing. The results indicate that most ARGs were evenly distributed along the transportation without evident attenuation, revealing a constant potential risk posed by sewerage in disseminating antibiotic resistance across the city. Sul2 gene was an order of magnitude higher than other ARGs. Moreover, besides the variation in different catchments, the ARGs profiles showed obvious seasonal rules, which is correlated with the discharge of different antibiotic categories. Furthermore, the correlation between the amount of intI and ARGs was significant (R^2>0.8) in August 2016 and October 2015, but not in other months, possibly implicating the ARGs transfer not only relied on the MGEs, but also influenced by environmental factors. The findings increase our understanding of sewerage as hotspots of ARGs, and contribute to prevent ARGs dissemination along sewers.

346A - Waste not the water: using municipal wastewater for community-scale health assessment

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Municipal wastewater is emerging as an ideal composite matrix for monitoring human and environmental health phenomena on a community scale, and has already proven useful as a means of providing community-scale information on human exposure to licit and illicit drugs. While very much an emerging field, wastewater monitoring could also be used to deliver valuable community health information such as early-onset warning of infectious disease outbreaks and troublesome evolutionary trends in human pathogenic microbes. Here, we investigated antibiotic resistance and pathogen profiles in wastewater microbial communities from different municipal sources, including wastewater from hospitalized patients (on a ward by ward basis), general suburban wastewater, and wastewater treatment plant influents with differing degrees of residential/commercial/industrial inputs. We used a combination of high-throughput DNA sequencing for microbial community analysis and quantitative methods (digital droplet PCR, qPCR) for resistance/virulence gene and (opportunistic) pathogen analysis. We have also conducted phenotypic screening (resistance profiling) of selected opportunistic pathogens and antibiotic resistant bacteria (*Acinetobacter*,

Klebsiella, *Pseudomonas*). Results showed that important resistance genes such as *NDM, oxa-48, VIM, SME* and *qnrS* were significantly more abundant in municipal wastewater treatment influents than in hospital-specific wastewater sources. Targeted resistance gene analysis indicated that problematic and emerging resistance threats, such as methicillin resistant *Staphylococcus aureus*, KPC-producing bacteria, and plasmid-mediated resistance to colistin are already widespread in community wastewater, and in some cases putatively more prevalent in the general community than in hospitalized patients. This research gives insight into the opportunities and complexities of using municipal wastewater for microbial risk surveillance.

347A - Manure-soil interlayer: bacterial interaction of two different niches and their tetracycline resistomes

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The study of antibiotic resistance in agroecosystem soils is becoming relevant in recent years. Manure is considered as an important source and stimulator that contributes to enrichment of antibiotic resistome of the agricultural soils. An interface manure-soil layer represents specific soil habitat to study interactions among manure and soil bacteria and their resistomes.

We have studied bacteriome and its tetracycline resistome changes in a manure-soil interlayer under control laboratory conditions. A hot-spot experimental model was designed to separate biotic and abiotic effect of manure and soil in the interlayer. The model included control soils; control manure; soil treated with nutrients; soil treated with manure; gamma irradiated soil treated with manure. Selected tetracycline resistance genes, representing efflux pumps, ribosomal protection and enzymatic degradation, were screened and quantified in cultivable and total bacteriome. Sanger sequencing and NGS of 16S rRNA were used to identify the resistant bacterial isolates and to evaluate composition of bacterial community in the studied soil-manure interlayer.

Our results showed an enrichment of tetracycline resistome and its hosts in the manure-soil interlayer. The stimulation effect of manure nutrients on the soil resistance was not confirmed. We detected an inhibition effect of soil resident communities on antibiotic-resistant bacterial communities from manure. This study provided new information on ecology of antibiotic resistance in specific microhabitat of agricultural soil.

348A - Prediction of the intestinal resistome by a novel 3D-based method

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The intestinal microbiota is considered to be a major reservoir of antibiotic resistance determinants (ARDs) that could potentially be transferred to bacterial

pathogens. Yet, this question remains hypothetical because of the difficulty to identify ARDs from intestinal bacteria. Here, we developed and validated a new annotation method (called pairwise comparative modelling, PCM) based on homology modelling in order to characterize the Human resistome. We were able to predict 6,095 ARDs in a 3.9 million protein catalogue from the Human intestinal microbiota. We found that predicted ARDs (pdARDs) were distantly related to known ARDs (mean amino-acid identity 29.8%). Among 3,651 pdARDs that were identified in metagenomic species, 3,489 (95.6%) were assumed to be located on the bacterial chromosome. Furthermore, genes associated with mobility were found in the neighbourhood of only 7.9% (482/6,095) of pdARDs. According to the composition of their resistome, we were able to cluster subjects from the MetaHIT cohort (n=663) into 6 "resistotypes". Eventually, we found that the relative abundance of pdARDs was positively associated with gene richness, but not when subjects were exposed to antibiotics. Altogether, our results support that most ARDs in the intestinal microbiota should be considered as intrinsic genes of commensal microbiota with a low risk of transfer to bacterial pathogens.

349A - Characterization of *escherichia coli* resistant to antibiotics isolated from clinical environment of the city of San Juan de Pasto, Nariño-Colombia

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Bacterial resistance to antibiotics is one of the biggest problems that is faced worldwide. One of the bacteria with the greatest concern at the hospital level is *Escherichia coli*, a pathogen that has developed highly efficient mechanisms to acquire, store and distribute genes associated with resistance, acting as a reservoir of genes distributed in the community.

In the city of San Juan de Pasto, strains of *E. coli* have been reported, with marked resistance to beta-lactam antibiotics that cause an increase in the frequency of infectious diseases that are difficult to treat. However, there is little knowledge about its characterization and the determinants of resistance against the main antibiotics used in the medical field, therefore, this study aims to characterize *E.coli* as a first step to elucidate more clearly how they are generating these transfer processes.

Initially in this study a description of the macro and microscopic characteristics of 16 clinical isolates was carried out, subsequently their biochemical characteristics were determined using specific tests for Gram negative bacteria, the resistance profile to different antibiotics was evaluated and finally its molecular characterization was carried out employing chromosomal DNA extraction, restriction enzyme cutting and plasmid profile determination. It was determined that all the isolates had similar macroscopic characteristics and a bacillary morphology characteristic of the *E.coli* bacteria. Through the correlation of molecular and phenotypic parameters, it was established that all isolates were resistant to beta-lactam antibiotics, aminoglycosides and fluoroquinolones, some of which were related to the presence of plasmids.

350A - Potential reduction of antibiotic resistance proliferation in anaerobic membrane bioreactor (AnMBR) microbial communities

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Antibiotic resistant bacteria and antibiotic resistance genes are an emerging concern in wastewater treatment plants and their effluents. Anaerobic membrane bioreactors (AnMBRs) are a new treatment technology that combine energy recovery via biogas production and membrane separation. Relative to aerobic treatment systems, antibiotic resistance proliferation may be mitigated due to significantly lower biomass yields and the anaerobic biochemical environment reducing horizontal gene transfer and ultimately alleviating concerns with effluent reuse and biosolids land application. The purpose of this study was to determine the abundance of antibiotic resistance genes and other mobile genetic element markers in AnMBR systems. To achieve this, a bench-scale AnMBR was operated using submerged ceramic microfiltration membranes. Biomass and effluent samples were taken weekly for resistance gene and mobile genetic element analysis using quantitative PCR. High-throughput sequencing was also used to evaluate microbial community structure.

Results showed that an erythromycin resistance gene (*ermF*) was the most abundant resistance gene detected in suspended biomass, with an average copy number of 0.17 ± 0.05 *ermF*/16S rRNA gene. *ermF* abundance in membrane biofilm biomass was significantly lower at 0.04 ± 0.01 *ermF*/16S rRNA gene. Conversely, a tetracycline resistance gene (*tetW*) was significantly more abundant in biofilm biomass as compared to suspended biomass at 0.004 ± 0.0003 and 0.001 ± 0.0003 *tetW*/16S rRNA gene, respectively. Our findings suggest that membrane biofilms may play an important role in ARG attenuation and perhaps removal. Ongoing work is characterizing antibiotic resistance profile response to increasing concentrations of sulfamethoxazole and other antibiotics.

351A - Genetic diversity and antimicrobial resistance genes of *Campylobacter jejuni* and *C. coli* in West Africa

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Campylobacter jejuni and *C. coli* are among the most common causative agents of gastroenteritis globally. However, the genetic diversity of these species in Africa is still poorly known. In 2009, we isolated campylobacters from the faeces of under 5-year-old gastroenteritis patients from Burkina Faso. From 31 isolates analyzed by Multi-Locus Sequence Typing (MLST) we detected 28 different sequence type (STs), of which 21 were novel. In the recent Whole Genome Sequencing (WGS) analysis of 40 of the isolates, they appear to belong to two large global ST complexes, a large chicken-associated complex and a human-only cluster. The *C.*

jejuni isolates are dispersed between the two European clusters of *C. jejuni* and the Asian isolates, whereas for the *C. coli* isolates there is distinct clustering by continent. The isolates commonly carry *bla*OXA-61 and *tet*(O) resistance genes, but no resistance genes against erythromycin or ciprofloxacin used for treatment of campylobacter infections.

To better understand the full diversity present in West-African campylobacters, we initiated a large-scale sampling in Burkina Faso and Ghana, including human, animal and environmental samples, in 2017. We aim to collect 1000 isolates and will use WGS analyses to understand the genetic diversity and evolution of the West African isolates and how they relate to the isolates from the other continents. Sampling multiple animal and environmental sources will help us to infer the transfer routes of antimicrobial resistance genes to humans. In the poster we will present the interim results of this large-scale study.

352A - Antimicrobial resistant bacteria in soil microbiomes

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In the present study we aimed to identify bacteria able to grow in the presence of several antibiotics including the ultra-broad-spectrum antibiotic meropenem in a British agricultural soil, by DNA stable isotope probing (SIP). Soil samples were incubated for four days with cefotaxime, meropenem, ciprofloxacin and trimethoprim in ^{18}O-water. Incubations were performed at 180 rpm, room temperature and in dark. Repeated addition of the antibiotics were added at the second day of the experiment. Controls without antibiotics and incubated in unlabeled water were also performed. The DNA was extracted and subjected to isopycnic centrifugation to identify microorganisms enriched with ^{18}O. We analysed the abundance of bacterial 16S rRNA genes using qPCR. The V4 region of the 16S rRNA gene from the “heavy” and “light” fractions were sequenced using Illumina MiSeq. After incubations, we detected an increase of the 16S rRNA copy numbers in the “heavy” fractions of the treatments with labelled water compared with their controls. We also detected that the treatments resulted in differences in the community composition of bacteria on the level of operational taxonomic units (OTUs; 97% sequence similarity). Results show that members of the phyla Acidobacteria are highly abundant after two days of incubation with antibiotics. We also observe the presence of several Proteobacteria including *Stenotrophomonas* after four days of incubations. The results indicate that both non-pathogenic soil- dwelling bacteria as well as potential clinical pathogens are present in this agricultural soil, but it is still unclear if horizontal gene transfer between these groups can occur.

353A - Sewage from airports exhibits high abundance and diversity of antibiotic resistance genes

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Sanitary facilities of airplanes and airports are shared by an international audience. We hypothesized the corresponding sewage to be an extraordinary source of antibiotic resistant bacteria and resistance genes in terms of quantity and diversity. We tested the hypothesis by complementary approaches: metagenomics, quantitative PCR and cultivation. Using these, we analyzed resistance genes and antibiotic resistant bacteria in sewage from airplanes, terminals, and municipal treatment plants receiving different loads of airport-borne sewage. As expected, airplane sewage contained a rich set of antibiotic resistance genes in high relative abundance, many of which (237) are located on mobile genetic elements. Moreover, combined resistance against third generation cephalosporins, fluorochinolones and aminoglycosides was exceptionally likely (28.9%) among *E. coli* isolated from airplane sewage. This percentage exceeds the one reported for German clinical isolates by a factor of 8. We conclude that sewage from airports can effectively contribute to the quick and global spread of antibiotic resistance.

354A - Ecological role of plasmids in Arctic permafrost environments

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Permafrost-affected soil is characterized by a low biomass, spatial heterogeneity and strong environmental fluctuations. The thawing of high Arctic permafrost releases nutrients potentially leading to higher microbial activity. These environmental conditions have been suggested to create a “hot spot” for plasmid exchange. Plasmids have the potential to rapidly transfer genetic traits within a bacterial community. Plasmid diversity and the plasmid-mediated plasticity of soil bacteria are studied by metagenomic sequencing, pure isolates or transformed bacterial cultures. Our knowledge about the role of plasmids in the adaptation of microbial communities to changing environmental conditions in the Arctic is still limited.

In this study, the cultivable fraction of active layer permafrost soil was investigated. We applied structural and functional annotations of sequenced metaplasmidomes from two sites of Samoylov Island, Siberia. This was combined with 16S rRNA gene sequencing, qPCR, and PCR replicon typing for the identification of plasmid incompatibility groups.

The metaplasmidome analysis suggests that the original hosts of the plasmids are bacteria of the genera *Acinetobacter*, *Pseudomonas*, *Serratia* and *Janthinobacterium*. The taxonomic diversity of potential plasmid hosts does not resemble the bacterial diversity of the full environment. Sequenced plasmid genes show high potential for mobilization (the presence of *tra* genes), microbial stress tolerance (multidrug efflux systems [SMR, RND], heavy metal resistance genes [e.g. *CzcD*, *TerC*], and UV resistance systems. These traits suggest an important role of plasmids in the adaptation of certain bacterial taxonomic groups to harsh changing environmental conditions in the Arctic.

355A - Quantitative and qualitative response of class 1 integron to different antibiotic stresses

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Class 1 integron (*intI1*) plays an important role in the spread of antibiotic resistance genes (ARGs). However, little is known about the response of *intI1* in environmental microbial community to different antibiotics' stresses. In this study, we constructed four aerobic biofilm reactors, three of which were spiked with increased antibiotics (streptomycin, oxytetracycline, and spiramycin) concentrations (0, 0.1, 1, 5, 25 and 50 mg/L) and one as control. *IntI1* was enriched with the increase of streptomycin and oxytetracycline concentrations, but no increases in its abundances were detected in spiramycin and control systems, indicating *intI1* did not quantitatively positively respond to all types of antibiotics. With the increase of streptomycin concentration from 0 to 50 mg/L, the proportion of aminoglycoside ARGs cassettes increased from 22.4% to 57.4%, and cassette arrays containing aminoglycoside ARGs became prevalent, suggesting *intI1* might directly contribute to the horizontal dissemination of aminoglycoside ARGs. On the other hand, though *intI1* showed significant correlation with three abundant tetracycline ARGs (*tetA, tetC* and *tetG)* ($p < 0.01$) in oxytetracycline system, no *tet* genes were found as gene cassette in *intI1*. Further analysis of the flanking regions of *intI1* through bacterial isolation and genome sequencing, *intI1* was found to be syntenic with *tetA, tetC* and *tetG* genes in chromosome or plasmids, indicating co-selection contributes to the enrichment of *intI1* under oxytetracycline stress. This study provides a comprehensive insight into the response of *intI1* to different antibiotic stresses, which is helpful for the management and control of antibiotic resistance in the environment.

356A - Antibiotic resistance activity, genetic mobility and host pathogenicity in the Swiss wastewater and its receiving river ecosystem

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Wastewater is an important point source of antibiotic resistance and pathogenicity determinants into the environment, thus the activity, genetic mobility, and host pathogenicity of resistance genes released are key aspects for their risk assessment.

Here we used quantitative metagenomics and metatranscriptomics to achieve a broad-spectrum view of differential expression activities of ~20 antibiotic classes from wastewater influent to effluent. Clinically important resistance genes of pathogenic origins and flanked with mobility elements, such as extended-spectrum beta-lactamases and carbapenemases (ESBL), are widespread in the wastewater effluent with surprisingly high relative abundance and transcriptional activities, revealing a great potential for resistance dissemination in the receiving rivers. The sub-inhibitory level of wastewater-borne macrolides is revealed to select macrolide and vancomycin resistance. In particular, the contig-based analysis reveals

considerable co-localization between resistance and mobility genes and implies a history of substantial horizontal resistance transfer involving human bacterial pathogens in wastewater. Based on cultivation, flow cytometry, and matrix-assisted laser desorption ionization biotyping, we investigate the prevalence of ESBL- and carbapenemase-producing bacteria in the upstream and downstream rivers (n=11). Their abundance was more affected by precipitation events than wastewater input. However, isolates were mostly indigenous plant root and/or soil *Pseudomonas* and *Stenotrophomonas* spp., although opportunistic pathogens are identified.

In conclusion, wastewater effluents and their receiving rivers harbor bacteria in which antibiotic resistance can be highly mobilized, expressed, and pathogenic. We propose the use of mobility incidence (M%), host-pathogenic incidence (P%), and contig-divergence incidence (D%) as standard parameters for quantitative risk-assessment models of antibiotic resistance in various ecosystems.

357A - Evaluation of the effect of wastewater irrigation on the prevalence of ARGs in surface soil and subsoil passages

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Conventional wastewater treatment effluents are containing several emerging contaminants; such as antibiotic resistance genes (ARGs). To define the wastewater irrigation impact on ARGs abundance and prevalence, during wastewater irrigation in soil and subsoil passages, we sampled a field irrigated with treated wastewater, which is associated with the company “Abwasserverband Braunschweig”. The sampling procedure took place at the end of a three months period of rainfall irrigation and at the start of wastewater irrigation season with secondary treated effluent. The sampling field site was containing four lysimeters before and after irrigation, each of them connected to three depths (40, 80 and 120 cm). In addition we sampled the surface soil of the each lysimeter establishment. Using quantitative PCR, we analyzed eight ARGs (*bla*$_{TEM}$*, bla*$_{CTX-M32}$*, bla*$_{OXA}$*, bla*$_{CTX-M15}$, *tetM*, *bla*$_{KPC-3}$, *qnrS*, *sul1*), along with the genes *int1* and *16SrRNA*; and we estimated their abundance (copies/L) and relative abundance (copies/*16SrRNA*). The results showed a clustering of the tested genes in three groups: a) the genes that their abundance and relative abundance increased significantly during wastewater irrigation (*bla*$_{TEM}$*, bla*$_{CTX-M32}$*, bla*$_{OXA}$*, tetM*, *qnrS*), b) genes that only their relative abundance was increased significantly (*sul1* and *int1*) and genes that their relative abundance was not significantly different during wastewater irrigation season (*bla*$_{CTX-M15}$ and *bla*$_{KPC-3}$). Therefore, we conclude that the levels of prevalence for the majority of ARGs are affected by the wastewater irrigation leading to significant increase in the subsoil passages and the surface soil.

358A - Antibiotic resistance gene abundance in human impacted environments correlates with fecal pollution with no signs of selection

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Treated wastewater discharge releases antibiotic resistant bacteria and resistance genes to natural environments. Concerns about selection and dissemination of resistant bacteria and resistance genes in environments receiving treated sewage has been raised. However, the persistence, faith and possible transport of these bacteria and genes in the environment is not well understood. It is speculated that antibiotics and other selective agents co-released in the effluents could select for resistant bacteria and/or promote the dissemination of the resistance genes often located on mobile genetic elements. Class 1 integrons have been used as a proxy for anthropogenic impact with good resolution. They carry several different resistance genes and are also subjected to selection themselves. Therefore, class 1 integrons cannot be used to independently determine selection and dissemination of ARGs in environments with anthropogenic impact. We adopted a method using markers for fecal pollution to study selection in polluted environments using public metagenomes and showed that in several sewage effluent polluted environments antibiotic resistance gene abundance follows fecal pollution levels and did not observe any signs of selection or wide-scale dissemination of antibiotic resistance. On the other hand, signs of selection could be detected in sediments polluted with waste waters from pharmaceutical industry containing high concentrations of antibiotics. Our results bring insight to the dynamics of ARGs in sewage receiving environments and highlight the importance of using markers which are not affected by selection for example by tracking fecal pollution when assessing the selection and dissemination patterns of antibiotic resistance genes in the environment.

359A - Diverse triclosan resistant genes revealed by triclosan resistome analysis in soil

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Triclosan, a synthetic antimicrobial used in a variety of consumer products, targets the highly conserved Enoyl-ACP reductase, thereby blocking bacterial fatty acid biosynthesis. Resistance to triclosan is considered to be associated with its extensive use, but the distribution of triclosan resistance has not been well characterized. We utilized a functional metagenomics based screening of the soil metagenome, which revealed that triclosan resistance is mediated by a variety of target enoyl reductase homologs, some efflux pumps and other candidate hypothetical proteins. The 7-α-HSDH-like, FabG-like, or the unusual YX7K-type Enoyl-ACP reductase conferred significant triclosan resistance and were prevalent in human pathogenic bacteria, such as *Helicobacter* and *Campylobacter* species and other obligate intracellular pathogenic microorganisms. Additionally, multiple resistance to other antibiotics was found to be conferred by antibiotic resistance genes co-localized with triclosan resistance determinants. Also, genome-wide analysis of 231 major pathogenic bacteria revealed that potential triclosan resistance determinants were abundant among those pathogens. Further, microbiome analysis of 14 different environments has revealed that both the prototypic and metagenomic triclosan-resistant Enoyl-ACP reductases are highly diverse in nature and potentially triclosan-resistant pathogenic bacteria were prevalent in some of those environments. Additionally, selective enrichment of triclosan-resistant specific Enoyl-

ACP reductases was observed in presumably triclosan-contaminated environments with reduced Enoyl-ACP reductase diversity. Our results suggest that triclosan-resistance determinants are diverse in nature, and long-term extensive use of triclosan can lead to the selective enrichment of triclosan-resistant bacterial pathogens, possibly with additional resistance to multiple antibiotics, in natural environments.

360A - Evolution under metal stress increases the plasmid uptake ability of microbial communities

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Heavy metal cations are consistently used in pig feeding and consequently enter soils through manure application. During both processes the microbial communities are evolving under metal stress conditions. Plasmid transfer is considered an important adaptive mechanism that can provide bacteria with resistance determinants against many environmental stressors. However, it is not known if evolution under stress conditions itself can directly stimulate this process.

We, therefore, evaluated, if evolving pig fecal and soil microbial communities under metal stress, changes their permissiveness to plasmids in the presence and absence of metal stress.

In experimental evolution experiments we evolved both communities under exposure to CuSO4 for 2 (fecal) or 6 (soil) weeks parallel to non-exposed controls. After evolution the community as well as single isolates were mated with a *mCherry*-tagged donor carrying the zygotically inducible *gfp*-tagged plasmid pKJK5 in presence and absence of copper. Transconjugants were quantified using advanced microscopy.

The pre-evolved communities increased their plasmid uptake under metal stress during the matings. However, the imposed metal stress lowered the plasmid transfer frequency for the non-stressed control communities. While both communities took up significantly more plasmids under their pre-exposed conditions, plasmid uptake under metal stress for the pre-exposed communities was significantly higher than for the non-stressed control in absence of metals. Thus, evolution under metal stress increased their general plasmid uptake ability, if exposed to metal stress. Further, isolates that were evolved under metal exposure displayed a higher likelihood of plasmid uptake based on phylogenetic distance to the donor, than their non-exposed counterparts.

361A - Fate of antibiotic resistant bacteria in two different constructed wetland designs

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Horizontal Gene Transfer (HGT) of Antibiotic Resistance Genes (ARG) in aquatic environments drives the development of novel Antibiotic Resistant Bacteria (ARB). This transfer can be substantial in wastewater treatment plants, where diverse microorganisms and various types of environmental stress are found.Constructed Wetlands (CWs), near-natural wastewater treatment options, can serve as model systems to illuminate more clearly the driving forces for HGT of ARG in aquatic ecosystems. Towards the aim of identifying such forces, we enumerated selected ARB, ARG, and class 1 integrons by culture-independent and culture-dependent methods in two horizontal-flow CWs receiving pre-treated municipal wastewater. One CW was aerated and the other was not. In addition, bacterial community profiling was carried out via 16S amplicon sequencing, and water samples were also analyzed for conventional wastewater parameters and antibiotics. Results showed a significant attenuation of resistance indicators in the effluent of both wetlands. However, in the aerated CW the relative abundance of some ARG and class 1 integrons increased along the flow path, which is an indicator for HGT. In the same CW, the abundance of cultivable Escherichia coli declined more steeply than their numbers based on molecular methods, which suggests that oxidative stress was acting on cells. There are two main conclusions: (1) CWs are a suitable technology to remove ARB/ARG from wastewater, with aerated systems performing better in terms of absolute abundance attenuation; (2) aeration could constitute an important stress factor, triggering an increase in HGT of some ARG, depending on their genetic context.

362A - Prevalence and antibiotic resistance mechanisms of staphylococcal bacteria isolated from meat and dairy products in Irene,South Africa

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Staphylococcus aureus has been reported as major pathogens in livestock.The pathogenic potential of *S.aureus* is attributed to a combination of extracellular factors and invasive properties that are controlled at genomic level.Livestock-associated strains may evolve on farms because of the use of antibiotics in animal husbandry.These antibiotics may be used as feed additives for growth promotion in industrial livestock and poultry,for prevention of disease within a herd,or for treatment of existing disease outbreak.Agricultural -use antibiotics may include many classes that are also relevant for human health,including tetracycline,macrolides,penicillins and sulfonamides,among others.Antimicrobial resistance generated during animal husbandry may then be spread to the general human population on a number of different manners including (i)contact with contaminated meat/milk products (ii)occupational contact (farmers,meat packers,butchers,etc) and (iii)potential secondary spread into the larger community from those who are occupationally exposed.The aim of the study was to employ

molecular techniques to identify the following A.aureus pathogenic factors: Protein A (Spa);Capsular Polyacrylamide (*Cap5*);Exfoliative toxins (*Eta*), α-Hemolysis (*Hla*);Thermonuclease (*Nuc*), Toxic shock syndrome (*TssT*) and Enterotoxins (*sea,seb,sec,sed*). Furthermore,to investigate antibiotic resistance genes in the *S.aureus* isolates. Amongst the classes of antibiotics,macrolides-lincosamide-streptogramin B (MLSB) were used.

363A - Characterization of Anthropogenic Impact to the Antibiotic Resistome of Han River using Metagenomics and Culture Collection

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There is growing interest in understanding the broad ecology of antibiotic resistance. In the field, evaluation of the impacts of anthropogenic activities on the environmental resistome is an emerging issue. To address this issue, we analyzed antibiotic resistome in our Han River sampling series, which span from pristine to urban area. We obtained data from high-throughput qPCR array, metagenome sequencing, and culture collection of antibiotic-resistant bacteria to determine how the resistome, mobilome and bacterial taxa of river change under increasing anthropogenic pressure. Blooming of antibiotic resistance genes was observed from downstream, densely populated area. Human population density and various indicators of anthropogenic pollution were significantly correlated with the abundance of resistance genes. Comparison of resistome compositions revealed that the downstream samples were distinct and functionally richer than the upstream samples. This trend was not observed from the taxonomic compositions or the overall gene contents of microbial communities. Downstream resistomes exhibited higher similarity to the human gut resistomes than upstream resistomes did. Bacterial families *Aeromonadaceae*, *Enterobacteriaceae*, *Microbacteriaceae*, *Moraxellaceae and Pseudomonadaceae* stood out as dominant contributors of riverine resistome by taxonomic classification of metagenome contigs and culture collection. In *Aeromonadaceae* strains, downstream isolates displayed stronger resistance phenotype, in line with the increase of *Aeromonadaceae*-associated resistance genes in the downstream metagenomes. Antibiotic-resistant genes were frequently associated with mobile genetic elements, and the families *Enterobacteriaceae* and *Moraxellaceae* appeared to be the taxa most actively involved in the exchange of antibiotic resistance genes. Our data exemplifies the significance of anthropogenic impacts on the riverine resistome.

364A - A single-cell microfluidic chip to observe and quantify transfer of antibiotic resistant plasmid in bacterial community under antibiotic stress

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Horizontal gene transfer is considered as the major way for spreading of antibiotic resistance, and there is a rising concern that antibiotics can promote horizontal

transfer of antibiotic resistance genes. However, the previous studies suggest a lack of conclusive evidence supporting this notion in general, largely due to the lack of well-defined quantitative experiments to address this question. In this study, a single-cell microfluidic chip was introduced to observe and quantify transfer of antibiotic resistant plasmid. Bacterial community extracted from activated sludge was used as recipients, while *Escherichia coli.* carrying with the antibiotic resistant plasmid pKJK5 was used as the donor. Tetracycline, trimethoprim and amoxicillin as typical antibiotics were tested their effects on plasmid transfer. Bacterial cells grew as a monolayer in the chambers, facilitating tracking the transfer processes and identifying the change of trans-conjugants at real time. The results showed that horizontal gene transfer mostly happened when the recipient bacteria divided, and vertical gene transfer played an important role in the spread of antibiotic resistant genes. Under the similar inhibition effect on donor strains, tetracycline didn't promote transfer frequency while amoxicillin did, probably because amoxicillin can make bacterial cells elongation. While donor strains could grow well under trimethoprim stress, though it can inhibit the recipients at some extent, the transfer frequencies still increased. Therefore, the impact of antibiotics on horizontal gene transfer mainly depended on their effects on cell growth and morphology.

365A - Transfer and long-term persistence of plasmids encoding antimicrobial resistance in wastewater treatment plant microbial communities

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There is increasing concern that wastewater treatment plants (WWTPs) may contribute to the dissemination of antimicrobial resistance genes (ARGs). Indeed, there transfer of plasmid-encoded ARGs might occur as human intestinal and environmental bacteria mix. Assuming that most enteric bacteria die off, ARG survival depends on transfer to and maintenance in WWTP adapted bacteria. Here, we explore, at the community level, the notion that plasmids cannot maximize both their within-population maintenance and their transfer proficiency, but that a tradeoff exists between these two properties.

We provide the first assessment of the transfer and maintenance of typical ARG-carrying plasmids in a WWTP activated sludge microbial community. This community was challenged with *Escherichia coli* carrying one of three GFP-tagged plasmids spanning multiple important incompatibility groups: pKJK5 (IncP), R27 (IncH) and R64 (IncI). Transconjugants were separated by fluorescence-activated cell sorting and identified by 16S rRNA gene amplicon sequencing. pKJK5 transferred to a broad phylogenetic range of bacteria, spanning 13 phyla, while transfer of R27 and R64 was limited to *Enterobacteriacaea*. Following initial transfer, the communities were serially propagated over at least 60 generations, and plasmid persistence monitored. For pKJK5, high plasmid-carrying fractions (up to 10%) were transiently observed, which decreased and remained above 1% by the end of the experiment. In contrast, R27 and R64 never reached high incidence. The description of the diversity of the plasmid carriers along the serial propagation experiment is

pending, but will provide a unique insight into the dynamics of contrasting plasmids in complex microbial communities.

366A - Characterization of antibiotic resistant bacteria in tropical urban watersheds

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Tropical freshwater urban watersheds receive regular rainfalls increasing the likelihood of contamination from urban runoffs. As urban watersheds serve as water sources for densely populated cities and antibiotic resistance is not commonly studied, we aimed to understand the prevalence of antibiotic resistant bacteria in the watersheds. We studied the heterotrophic plate counts of R2A agar supplemented with Erythromycin, Lincomycin, Kanamycin, Norfloxacin, Sulfanilamide or Tetracycline among six watersheds (R1, R2, R3, R4, R9 and R10) and compared their relative abundances (R2A with antibiotics/R2A without antibiotics) using non-parametric tests. Principal Coordinate analysis showed that watershed samples can be clustered into three broad groups (A to C) ($p<0.05$; SIMPROF) with Lincomycin showing the highest variation. Lincomycin resistance correlated positively with Group C samples while Group A samples correlated positively to one or more of the other five antibiotics. Group B samples tended to have samples with lower abundances of the six antibiotics compared to samples of the other groups. We identified eight taxa resistant to eight or more antibiotics of which four were categorized as potential human pathogens (*Pseudomonas, Acinetobacter, Stentrophomonas* and *Enterobacter*), and four were categorized as environmental bacteria (*Flectobacillus, Herbaspirillum, Acidovorax* and *Novosphingobium*). The prevalence of these 8 antibiotic resistant taxa were ascertained from amplicon 16S rRNA gene sequencing of 12 watersheds and data showed 4 watersheds with samples of higher relative abundances of these bacterial taxa. In this study, the water quality of urban watersheds were characterized based on identifying and estimating abundances of several bacterial taxa via high-throughput sequencing.

367A - Catalogue of antibiotic resistome and host-tracking in drinking water deciphered by a large scale survey

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Excesses of antibiotic resistance genes (ARGs), which are regarded as emerging environmental pollutants, have been observed in various environments. However, ARGs harbored in drinking water remain largely unexplored. In this study, we aimed at establishing an antibiotic resistome catalogue in drinking water samples from a wide range of regions and to explore the potential hosts of ARGs.

A catalogue of antibiotic resistome in drinking water was established, and the host-tracking of ARGs was conducted through a large-scale survey using metagenomic

approach. The drinking water samples were collected at point of use in 25 cities in mainland China, Hong Kong, Macau, Taiwan, South Africa, Singapore and the United States. In total, 181 ARG subtypes belonging to 16 ARG types were detected with an abundance range of 2.8×10^{-2} to 4.2×10^{-1} copies of ARG per cell. The highest abundance was found in northern China (Henan Province). Bacitracin, multidrug, aminoglycoside, sulfonamide and beta-lactam resistance genes were dominant in drinking water. Of the drinking water samples tested, 84% had a higher ARG abundance than typical environmental ecosystems of sediment and soil. Metagenomic assembly based host-tracking analysis identified *Acidovorax, Acinetobacter, Aeromonas, Methylobacterium, Methyloversatilis, Mycobacterium, Polaromonas* and *Pseudomonas* as the hosts of ARGs. Moreover, potential horizontal transfer of ARGs in drinking water systems was proposed by network and Procrustes analyses.

The antibiotic resistome catalogue compiled using a large-scale survey provides a useful reference for future studies on the global surveillance and risk management of ARGs in drinking water.

368A - Microbial resistances in built environments are exacerbated by excessive microbial control

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Antimicrobial resistances are pushing humankind into a serious health crisis. This is of particular concern in the hospital environment. It is discussed, whether microbial control and enhanced cleaning procedures could impact the built environment microbiome, supporting the spreading of resistances.

To better understand such adverse anthropogenic impacts on the composition of the residing microbiome and resistome, we compared different built environments using genome and gene centric shotgun metagenomics and 16S rRNA gene amplicon analysis. Investigated built environment categories represented different levels of confinement and cleanliness including an intensive care unit, cleanrooms and unrestricted private and public buildings.

In total, 42 genomes of high quality and 91 plasmids were recovered from the dataset. Different built environment confinement and cleanliness categories were accompanied by distinct microbial compositions and functional capabilities. Increasing confinement from the surrounding environment was mirrored by a clear fingerprint of mainly human-associated Gram-negative bacteria capable of degrading xenobiotics from applied cleaning reagents, lower replication rates, comprising more offensive virulence factors as well as genes involved in multidrug

efflux to counteract fluoroquinolones, triclosan or elfamycins. In summary, we observed that significant (P = 1.8 * 10-7) reductions of the microbial diversity by 50% inside confined built environments were accompanied by a significant (P = 0.01) increase of resistances by 20%.

As microbial diversity and the diversity of resistances were shown to correlate negatively, we propose that increasing and stabilizing the beneficial microbial diversity in buildings by optimized maintenance procedures would counteract the spreading of antimicrobial resistances in critical built environments.

369A - The Use of epicPCR to Determine the Host Range of Antimicrobial Resistance Genes in Manure and Manure-Fertilized Soils in Finnish Dairy Farms

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Modern animal production relies on the use of antimicrobials, which increases the emergence of antimicrobial resistance. Storage and use of animal manure as a fertilizer is a potential route for the dissemination of antimicrobial resistance genes via horizontal gene transfer.

The use of antimicrobials in animal production is more restricted in the EU than elsewhere in the world. Especially in Finnish animal husbandry the antimicrobials are used predominantly for treatment of infections. It has been shown that antimicrobial resistance genes are abundant in manure and the abundance increases during the winter storage. Therefore, the storage may enhance the effect of manure application, which is a potential route to horizontally transfer the antimicrobial resistance genes in manure to soil bacteria. Harvested feed, in turn, is a potential route from soil bacteria to gut microbes in livestock. The question remains, what is the host range of these genes? Are the species pathogenic and are the genes transferred horizontally?

We use culture-independent epicPCR to investigate the host range of aminoglycoside, beta-lactam and tetracycline resistance genes in fresh and stored manure and soil after fertilization in two Finnish dairy farms. The method links the antimicrobial resistance gene to the phylogenetic marker gene together, which gives answers to aforementioned questions.

370A - The fate of antibiotic resistant genes in treated wastewater irrigated soils and crops

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The use of treated wastewater (TWW) for irrigation is becoming more prevalent and could help mitigate water scarcity, especially in arid and semiarid regions.

Wastewater tretament plants does not completely remove antibiotics, antibiotic resistance bacteria (ARB) or antibiotic reistance genes (ARGs), therefore, effluents discharged from these facilities may contribute to ARGs accumulation in TWW irrigated soils and crops. We hypothesized that TWW irrigation would increase the level of ARGs in the irrigated soil and crops due to selective pressure generated by residual antibiotic compounds, and to the introduction of ARGs and ARB. To test our hypothesis, we assessed the abundance of seven ARGs, class 1 integron and 16S rRNA encoding genes in 114 samples of TWW and freshwater irrigated soils and crops. The samples were collected during two consecutive seasons cultivating vegetable crops (cucumbers and melons), testing water quality (TWW and freshwater), soil types (sand, loamy sand and clay) and soil treatments (surface and subsurface drip irrigation and soil plastic cover). The results revealed that TWW contains a diverse array of ARGs in high abundance compared to freshwater. Yet, ARGs' levels in the irrigated soils and crops were low regardless of the irrigation water quality or soil treatments, while the levels differed among soil types. The results indicate that ARB and ARGs harbored in TWW may not persist in the irrigated soil and thus do not transfer to the crops, suggesting that the impact of TWW on the irrigated soil resistome might be negligible.

371A - Unravelling plasmidome distribution and interaction with its hosting microbiome

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Horizontal gene transfer and the plasmids serving as gene transfer agents play an immense role in microbial evolution. Here we present a comparative study of plasmidomes across adjacent and different microbial environments present in different individual rumen microbiomes. Our findings show that the rumen plasmidome display enormous unknown functional potential currently unannotated in available databases, nevertheless this unknown functionality is conserved and shared with published rat gut plasmidome. Moreover, the rumen plasmidome is highly diverse as compared to its hosting microbiome, across both similar and different rumen habitats, our analysis shows that its structure is shaped more by stochasticity than selection. Nevertheless, it is an active partner in its intricate relationship with the host microbiome. Together, they interact with their environment: as environmental conditions change, so do the microbiome and the plasmidome, the latter being less constrained into defined ecological niches as the former. This study presents a unique look at a plasmidome variation in its natural environment and its ecological role as an independent entity.

372A - Transfer of an integrative and conjugative element in *Pseudomonas* may be triggered by oxidative stress

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The integrative and conjugative element (ICE) ICE*clc* from *Pseudomonas knackmussii* B13 confers its host the ability to degrade 3-chlorobenzoate (CBA). ICE*clc* is stably integrated in the genome, but can be transferred to new recipient cells by conjugation. ICE transfer exclusively occurs from a small subpopulation of transfer competent cells and is initiated during stationary phase after growth with CBA. The aim of this work is to better understand the possible trigger(s) and the role of CBA that favor development of the transfer competence state. Our working hypothesis is that some cells accumulate biochemical damage during growth, which is sensed by the ICE and triggers its activation mechanism. The ecological relevance of this may be that the ICE tries to escape cells that are in bad shape in order to survive in a hopefully healthier cell.

As a measure for cellular damage, we tested whether oxidative stress correlates to ICE activation. An oxidative stress bioreporter was constructed placing the *gfp* gene under the control of the promoter P_{AHPC} in *Pseudomonas putida* and its functionality was successfully calibrated using UV-A, paraquat and hydrogen peroxide. Next we tested whether growth on CBA is more stressful for cells than succinate. Indeed, exponentially growing cells on CBA display more fluorescence from P_{AHPC} than succinate-growing cells. We will describe ongoing time-lapse microscopy experiments to record the life history of individual cells in order to determine whether higher oxidative stress during exponential phase correlates to later ICE activation.

373A - Study on antibiotic resistome and bacterial community in Indonesian river

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Water environments are known as one of the hotspots of antibiotic resistance and horizontal gene transfer between bacteria in human, animal and the environment. However, there is still lack of data on the status of antibiotic resistance in the environment in less developed countries. To fill this gap, our study provides the data of antibiotic resistome profiles in an Indonesian river from spring water to estuary. We use 384 assays on qPCR Array (Wafergen-TakaraBio) to detect and quantify antibiotic resistance genes (ARGs), genes associated with mobile genetic elements and housekeeping genes. We also analyze the amplicons of 16S rRNA genes (Illumina) to study the bacterial community in the river environments.

Our results show that hospital wastes and urban activities are the main source of ARGs in surface river water in Yogyakarta, Indonesia. The impact of animal farms on ARG profiles is different compared to the hospital and urban activities. Most of the ARGs are diluted in the estuary, however some are still detected especially that are associated with the mobile genetic elements. This may lead to the spread of ARGs in the environment which may disseminate back to bacteria in human and animals. Human activities are also impacted the bacterial community in surface river water. We propose that a research unit which focuses on the surveillance of

antibiotic resistance in the environment is required. Also, it is important to improve the antibiotic resistance awareness and the urge to implement the regulations on antibiotic use in human and animals in Indonesia.

374A - Horizontal transfer of plasmids and bacteriophages from broiler chicken microbiome to *Salmonella* Heidelberg

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The chicken gastrointestinal tract (GIT) harbors a complex taxa of microbes that play key roles in the health and disease status of the host. The cecum is the part of the GIT that carries the highest microbial densities, has the longest residence time of digesta in the GIT and is a vital site for urea recycling and water regulation. Importantly, the chicken ceca provides a rich environment for bacteria to horizontally transfer genes between one another via mobile genetic elements (MGE) such as plasmids and bacteriophages. In this study, we determined the MGE that can be transferred from chicken cecal populations to *Salmonella* Heidelberg and *Enterococcus faecalis*. We performed whole genome sequencing on isolates recovered after 0.5, 6, 24 and 48 h of incubation in ceca under microaerophilic conditions. We observed significant temporal changes in the presence/absence of bacteriophages for both *S*. Heidelberg and *E. faecalis*. Notably, we recovered an *S*. Heidelberg isolate that carried an IncB/O/K-like (~ 88 kb) plasmid with BlaCMY-2 gene that conferred resistance to 3rd generation cephalosporins. This plasmid shared significant DNA homology with the IncK2 plasmids recovered from *E. coli* strains, and was present in moderate copies (6 – 24 copies/cell) after 48 h incubation of the ceca and under ampicillin selection. Conjugation between *S*. Heidelberg and other bacteria could not be reproduced in the laboratory setting, however, the plasmid was stable for 50 generations with and without selection. These results suggest that IncB/O/K-like plasmids are capable of spreading from cecal flora to *Salmonella*.

375A - Microbes living in built in environment

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Horizontal gene transfer via plasmids can facilitate the spread of antimicrobial resistance (AMR) markers between microbes. It is however, unclear whether or not the built environment (BE) mediates this spread. In this study, 6 heavily used subway stations in Stockholm city were sampled at winter, autumn, spring and summer to monitor the seasonal variation influencing the diversity of plasmid associated bacteria and their functional genes. The collected swabs were cultured in nutrient medium with or without antibiotics and then plasmids were directly extracted from the pooled grown colonies and prepared for next generation sequencing. The final processed reads resulted in 14 replication genes and a group of backbone

plasmid genes such as conjugation, mobility and partitioning genes. A wide range of antibiotic resistant classes were predicted. We identified 51 circular plasmids associated with bacteria including *Staphylococcus aureus*, *S. epidermidis* and *Acinetobacter baumannii* as the most abundant species. Interestingly, Streptococcus oralis subsp.tigurinus was identified in low abundance as well as *Prevotella dentalis*. There was a significant correlation between plasmid functional genes, seasons and the number of daily passengers (ANOVA p-value: 0.024). These results indicate the contribution of BE on the diversity and abundance of bacteria and AMR genes. Skin and gut microbiota were detected with limited number to be considered as a threat in BE. This study is one of the first report of AMR describing plasmids distribution in BE which provide an interesting overview of the microbes shaping the BE areas in the capital of Sweden.

Poster Pitch

376A - Plasmids in gut environments

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Plasmids, extrachromosomal genetic elements, can be found in all kinds of organisms and environments. Within microbial populations, plasmids contribute to genetic diversity and microbial evolution. This relevance is reinforced by horizontal gene transfer, a widespread plasmids' ability that allows for genetic exchange between organisms of various taxonomic distances. The microbiome, with its plasmids, affects life on a bigger scale and plays a key role in ecosystem functioning. The current knowledge about plasmids is insufficient to understand their role and relevance in Nature.
Here, we extracted plasmids from microbiomes of different mammalian hosts, sequenced their plasmidome using Illumina Hiseq sequencing and analysed the factors shaping their distributions.
Animals differed widely in their plasmidomes and many plasmids were found only in one animal. Moreover, the plasmids differed in length, the presence/absence of genes enabling horizontal gene transfer and accessory functions. However, clustering of the animals by the plasmids detected in their gut microbiome was influenced by the animals' gut system. Also clustering by accessory functions which are potentially beneficial for the plasmid's microbial host was governed by the animals' gut system.
These findings indicate that environmental selection is a driving force at even smaller scale than the cell as a living entity itself.

377A - Minimal concentrations of zinc ion promoted conjugative transfer of kanamycin resistance gene mediated by a plasmid isolated from chicken manure

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The prevalence and spreading of antibiotic resistance genes (ARGs) in animal manure have raised the global concern. Heavy metals are also widely existed in livestock farms and their concentrations are usually below the minimal inhibitory concentration (MIC). However, mechanism that refers to heavy metals at very low levels (<<MIC) will impact the transfer of ARGs are still limited. In this study, we isolated a conjugative plasmid (pKANJ7, ~30 kb) carried with an aminoglycoside resistance gene (*aphA*) from chicken manure. Culture medium, mating time, pH and Cu^{2+}/Zn^{2+} were considered as factors that influence the conjugative transfer of pKANJ7. Results showed that the highest frequency of conjugative transfer was observed after 8 h in PBS (0.1 M, pH=7). However, the transfer frequency increased after 8 h in LB with the propagation of donor and recipient. Mildly acidic condition (pH=6) tended to promote conjugative transfer of pKANJ7 compared with weak alkaline conditions (pH=8). In addition, 0.125-0.5 μg/mL of zinc ion significantly promoted conjugative transfer. The expression of *aphA* increased 14-fold at the concentration of 0.5 μg/mL compared to the control. Other conjugative transfer related genes, such as genes encoding periplasmic protein, pilus protein, inner membrane protein, outer membrane protein and ATPase, were also up-regulated at least 2.4 times. However, low levels of Cu^{2+} inhibited both the transfer frequency and the expression of related genes. Our study provided the essential information for controlling the spreading of ARGs in environment.

378A - Evaluating the mobility potential of antibiotic resistance genes by long-read sequencing

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The current short-read sequencing approach cannot assess the mobility of antibiotic resistance genes (ARGs) and thus will overestimate health risk of ARGs. We assessed the linkage profile of ARGs and mobile genetic elements in a manure community enriched from Ceftiofur treated dairy cow by two long-read sequencing technologies: PacBio and Nanopore. We found many incomplete ARGs, indicating short-read sequencing overestimates ARG abundance. Multidrug resistance genes were dominant ARGs (> 62% of total ARGs) in the community, however, only 0.8–1.5% were associated with mobile genetic elements. This supports the view that most multidrug resistance genes are intrinsic and their primary function is not antibiotic resistance. Similarly, peptide and quinolone resistance genes were largely detected but only small proportions of them were linked to mobile genetic elements. Beta-lactam and macrolide resistance genes accounted for 2.3–4.6% of total ARGs but 57–71% of them were connected to mobile genetic elements. Considering that macrolide (erythromycin) and beta-lactam (ceftiofur) antibiotics were used for enrichment of the bacterial community, selection may have facilitated enrichment of ARGs linked with mobile genetic elements. Tens of linkages among multiple classes of ARGs, metal resistance genes, and mobile genetic elements were observed, showing that a single selection will lead to co-enrichment and co-spread of multiple resistance genes. Our results demonstrate that only a small portion of ARGs was linked to mobile genetic elements, and selection pressure may facilitate enrichment of mobile ARGs. Both long-read sequencing technologies are promising to evaluate health risk of ARGs.

379A - Hospital wastewaters contribute greater diversity of antibiotic resistance genes to sewage treatment plant effluents and the receiving river resistome

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Antimicrobial resistance has become a major concern for human and animal health, as therapeutic alternatives to combat against multi-resistance bacteria are declining. Although antibiotic resistance genes (ARGs) are a natural component of all environments, human activities have increase their prevalence and promoted the development of new resistance variants. Among pathways, sewage releases can be a major link between human gut and environmental microorganisms. Nowadays, the differences in the level of residues and ARGs in community versus hospital-associated sewage sources is well known. However, their relative and specific contributions to the environmental resistome has not been clearly delineated.

Here, we quantified the contribution of community- versus hospital- associated sourced ARGs to the receiving water resistome, and also the role of a conventional sewage treatment plant (activated sludge-STP) to reduce the dissemination of clinical relevant ARGs into the environment. To do this, we used HT-qPCR targeting 285 ARGs conferring resistance to major classes of antibiotics. Microbial community composition at different compartments was characterized by 16S rRNA gene amplicon sequencing. We show that although hospital sewage represents less than 2% of the sewage flow, it is the dominant source of ARGs diversity in downstream river sediments. Furthermore, we show that sewage associated ARGs and microbial communities often pass directly through the STP, without establish into the activated sludge floc. Finally, the secondary clarifier plays a critical role, dividing the microbial community between microorganisms with flocculation traits and organisms that do not tend to aggregate, which significantly influences bacteria found in liquid effluents.

380A - Constraints on horizontal gene acquisition in bacteria: comparing intralineage and intergeneric crosses

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Bacteria are thought to adapt to specific ecological niches in part by assembling “genome islands” specifying sporadically-useful functions, which then circulate to other lineages. The RecA-independent mechanisms involved in assembly remain obscure. We aim to understand the frequency and nature of events that contribute to

island assembly. A focus is the Immigration Control Region (ICR) of enteric bacteria, an island enriched with genes for variable sequence-specific restriction enzymes.

We developed a conjugal system to transfer chromosomal DNA within and between laboratory lineages and genera to characterize RecA-independent gene transfer. The basal intralineage events, in derivatives of *E. coli* K-12, replace very large (60 kb-2 Mb) segments of recipient DNA with donor sequence. In preliminary results with intergeneric crosses using as recipient a restriction-deficient laboratory derivative of *Salmonella enterica* sv Typhimurium LT2, we identify two distinct properties: an increased fraction of events are additions, and shorter segments predominate. As groundwork for these experiments, we determined genome sequences of two strains: the restriction-deficient strain (mainly LT2 with a segment from *S enterica* sv Agona) and our isolate of wild type *S*. Typhimurium LT2. Comparison of the LT2 genes that determine the restriction activities SenLT2I (LT, StyLT in the early literature), SenLT2II (SA, StySA) and SenLT2III (SB, StySB) with those of the restrictionless strain allowed us to identify mutations potentially responsible. We highlight the genetic composition SenLT2II (SA), which has not been reported before. Variation contributed by four inducible prophages of *S.* Typhimurium LT2 was also revealed.

381A - Local environmental conditions determine the acquisition of indigenous plasmids by Pseudomonad populations in grassland soil.

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The transfer of plasmids between bacterial hosts allows for rapid changes in phenotype and potential adaptation to abiotic conditions. With increased anthropogenic management of land, the biological components of soil are required to deal with increased chemical and physical stress. Whilst it has been well established that changes to land-management can alter the composition of communities, it is not clear how these abiotic drivers affect horizontal gene transfer. To test the effect of anthropogenic land-use change on the frequency of plasmid transfer, we situated our study in the long-term glass-land experiment Nash's Field, located in Silwood Park, UK. This multi-treatment experiment, established in the early 1990's, has defined treatments that have resulted in a marked difference in both the soil conditions, and biotic make-up (both above and below ground). We chose to select ubiquitous mercury resistance-encoding plasmids to eliminate selective pressure as a factor. By introducing *Pseudomonas putida* UWC1 into the field site, we were able to select for the isolates that had taken up a plasmid, thereby becoming mercury resistance, *in situ*. Soil samples were also brought back to the laboratory for exogenous isolation of plasmids. We sampled throughout summer 2018 and monitored abiotic factors. Our results indicated that the abiotic soil and weather conditions were significant drivers of plasmid transfer both *in situ* and *in vitro,* specifically pH, but the chemical treatments did not change the frequency of plasmid transfer. The strongest effect, however, was found in the legacy effects of Pseudomonad presence in the month preceding sampling.

382A - Third generation cephalosporin-resistant *Klebsiella pneumoniae* from clinical and wastewater sources

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Third generation cephalosporin-resistant *Klebsiella pneumoniae* is a health-care associated infectious agent, also found in the environment. Whether clinical isolates maintain their traits once in the environment, and may present a human-health threat through the dissemination of antibiotic resistance is the question addressed here.

With this aim, a group of 59 third generation cephalosporin-resistant *K. pneumoniae* wastewater (n=25) and clinical isolates (n=34) were characterized based on their phenotypic and genotypic resistance and plasmids content. The dissemination of antibiotic resistance through horizontal gene transfer was also assessed.

Multidrug resistance was observed in most wastewater (80%) and clinical isolates (94%). The extended-spectrum β-lactamase genes were more prevalent in clinical (47%) than in wastewater isolates (24%). The carbapenemase gene *bla*$_{KPC}$ was only detected in clinical isolates (15%), and *bla*$_{VIM}$ and *bla*$_{IMP,}$ as well as colistin resistance genes, *mcr-1* and *mcr-2,* were absent in isolates from both origins. All strains had at least one plasmid, and three plasmids per strain were more common in wastewater than in clinical isolates (24% *vs*. 12%). Preliminary results showed that all isolates transferred resistance to third generation cephalosporins to a host in conjugative assays.

These results indicate that clinically relevant bacteria once released in the environment might retain clinical relevant traits and contribute to the spread of antibiotic resistance genes.

383A - Effect of environmental ARG expression under selective pressure on ARG transfer to human microbiome bacteria

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The continuous increase of antibiotic-resistant bacteria (ARBs) in medical clinics is a major global health concern. Although the use of antibiotics for human and animal

treatment seems to be related to the spread of ARBs, antibiotic resistance dispersion is also a natural environmental process. Furthermore, several studies have documented the influence of anthropogenic activity on the environmental "resistome". Novel antibiotic resistance genes (ARGs) may emerge in the non-clinical environment and spread to the human microbiome, from where the probability of their transfer to human pathogens increases. Thus, we were interested in the environmental factors that influence the rate and probability of ARGs transfer from non-human microbiome bacteria to human microbiome bacteria in non-clinical environments. Here, we report on the influence of *in situ* transcription rates on the transfer of genes in soil. In order to determine whether the transcription of ARGs under different selective pressures increases their transfer rate to human microbiome bacteria, natural soils and soils incubated with antibiotics were inoculated with a labelled strain of *E. coli.* At different time points, these *E. coli* were isolated from soil microcosms by flow cytometry and a combination of metatranscriptomics and qPCR was used to evaluate the transfer of environmental ARGs to *E. coli* as a function of their transcription rate in the donor bacteria. This approach provided a model for the study of environmental ARG transfer to human microbiome bacteria directly in soil and highlights the importance of gene expression monitoring in risk assessment studies.

384A - Do wastewater antibiotic resistant bacteria survive in the presence of a healthy-human gut microbiome?

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Water reuse has been associated with the dissemination of antibiotic resistant bacteria and genes (ARB&ARGs) in the environment. The major question is how this contamination may threat the human health, since entry via food chain is a likely possibility. This study aims to assess whether the complex human gut microbiome may hamper the survival of known wastewater ARB.

Faeces-based microcosm assays were inoculated with wastewater ARB (*Escherichia coli* A2FCC14 and *Enterococcus faecalis* H1EV10) known to harbor the ARGs *blaTEM*, *blaCTX*, *blaOXA-A* and *vanA*, respectively. Assays were performed under aerobic and anaerobic conditions, with or without sub-inhibitory concentrations of cefotaxime and vancomycin. Assays were followed for at least 7 days, based on cultivation, quantitative PCR and bacterial community analyses.

Both strains survived in the presence of the faecal microbiota for a week and their ARGs could be detected and quantified at least for one month. While *Enterococcus faecalis* H1EV10 behaved similarly under aerobic and anaerobic conditions, *Escherichia coli* A2FCC14 had a sharper reduction anaerobically. The presence of sub-inhibitory concentrations of antibiotics did not affect the survival of the ARB. To have a better understanding of the effects that selective pressure may have on the autochthonous community composition, microbiome analyses are ongoing.

The main conclusion of this research so far, is that ARB with origin in treated wastewater are able to survive and are not outcompeted by the autochthonous microbiota until a week and their ARGs could be quantified alike.

385A - Antimicrobial resistance in soil microbes mediated by resistance evolution and horizontal gene transfer

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Soil microbes are exposed to different environmental stressors originating from various sources. Biocides used as material preservatives can represent environmental stressors since they are in direct contact with the environment including soil. Microorganism in soils can adapt to stress by different mechanisms; for example, by the evolution of resistance by de novo mutations or acquisition of resistance genes via horizontal gene transfer (HGT). Here, we hypothesize that material preservatives drive the evolution of biocide resistance enabling the potential for cross-resistance to antibiotics. Furthermore, we hypothesize that material preservatives cause increased frequencies of horizontal gene transfer (HGT, i.e. altered community permissiveness) facilitating microbial community adaptation to stress. We will culture soil microorganism with increasing concentrations of selected biocides followed by antibiotic susceptibility determination. Moreover, we will incubate soil mesocosms with selected biocides to investigate if these compounds promote HGT of plasmids that carry resistance genes in soil microbial communities. Together these results will elucidate the potential for the evolution of biocide resistance and cross-resistance to antibiotics as well as the effect of biocides on adaptation to environmental stressors in soil microbial communities.

386A - Are antibiotic resistance genes differentially abundant in chlorinated drinking water systems as compared to non-disinfected systems?

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Antimicrobial resistance (AMR) poses severe implications for clinical treatment globally. This issue is aggravated by misuse of antibiotics and anthropogenic release of bioactive compounds into the environment. The urban water cycle interfaces human activity and the environment, but components such as drinking water systems, that are in direct and prolonged contact with consumers, have received less attention than counterparts as critical points of AMR dissemination. Disinfectant residuals are used to manage microbial growth in distribution systems, however they may introduce additional health related concerns (e.g. disinfection by products), which prompted a shift towards non-disinfected systems in some European countries. In order to assess the current situation of AMR in the drinking water microbiome, chlorinated and non-chlorinated systems from England, Scotland, and Netherlands were sampled. Water quality parameters were recorded and samples were filtered to concentrate biomass for DNA extraction. Metagenomic

sequencing reads were co-assembled and followed by gene calling and annotation against CARD database with a stringent reciprocal BLAST criteria. Metagenomic sequencing indicates high diversity of AMR traits, with over 400 antibiotic resistance ontologies (ARO) across assemblies. Generally, AMR traits are more abundant in chlorinated samples. Data suggest a diverse and uneven resistome in both disinfected and non-disinfected samples, a statistically different resistome structure when grouped by strategy, and that AROs related to efflux resistance mechanisms dominate in both systems. On-going efforts using genome binning aim to contextualize these genes in terms of their host to determine their presence in opportunistic pathogens to estimate risks from a public health perspective.

387A - Towards the detection of horizontal gene transfer in metagenomics datasets

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Horizontal gene transfer (HGT) is thought to be an important driving force for microbial evolution and adaptation, including the development of antibiotics resistance and niche adaptation. Metagenomics provides an opportunity to study horizontal gene transfers on the level of microbial communities, however, analysis methods for this are currently lacking. Here, we developed three bioinformatics pipelines to aid the detection of horizontal gene transfer in metagenomics datasets. Firstly, Binning_refiner was developed to improve the quality of genome bins derived from metagenomics dataset through the combination of different binning programs. Our results demonstrated that Binning_refiner can significantly reduce the contamination level of genome bins and increase the total size of contamination-free ‘good-quality’ genome bins. Secondly, HgtSIM was developed to simulate HGT events among microbial community members with user-defined mutation levels. It was developed for testing and benchmarking pipelines for recovering HGTs from complex microbial datasets. And thirdly, MetaCHIP was developed to identify horizontal gene transfers at community-level through the combination of best-match and explicit phylogenetic tree approaches. Assessment of its performance on both simulated and real datasets showed that it can effectively predict HGTs with various degree of mutations from microbial communities. The results also showed that the detection of very recent gene transfers from metagenomics dataset is affected by the reads assemble step, as the genome background of recently transferred genes cannot be recovered with currently available assemblers. All pipelines are implemented in Python and are freely available at https://github.com/songweizhi.

388A - *In situ* detection of conjugative transfer of broad host range plasmids containing antibiotic resistance genes

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Antibiotic resistance genes (ARGs) are commonly found on conjugative plasmids that can transfer horizontally between bacteria. Environmental bacteria, such as the

rich communities found in water and wastewater treatment systems, represent reservoirs of ARGs that pathogenic bacteria may acquire antibiotic resistance from via horizontal gene transfer. Broad host range plasmids (BHRPs) have been attributed to the spread of ARGs in the environment. However, the propagation of BHRPs is not well understood in mixed communities such as those found in water and wastewater systems. In situ measurements of conjugative transfer of ARG-harboring plasmids are needed to understand the frequency of HGT and spread of ARGs in the environment.

Here, we demonstrate the use of gas-reporting biosensors that report on *in situ* conjugation events. We engineered BHRPs with a gene encoding a gas reporter and transformed the plasmids into several donor strains. The donor strains were engineered such that gas production only occurs after a conjugation event and the plasmid enters a recipient cell. Gas production in several recipient strains was characterized to understand differences in gas production across species. In addition, gas production by *E. coli* S17-1 containing the engineered BHRPs was characterized in several different matrices, including wastewater treatment plant influent and effluent. Detection limits of the gas-reporter are being explored. This research will enable us to obtain *in situ* measurements of conjugation and elucidate the impact of environmental and treatment plant operational conditions on ARG transfer rates in mixed communities.

Poster Pitch

389A - Broad host range plasmid can disseminate widely in natural soil microcosms

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Plasmid conjugation is one of the most dominant mechanisms of horizontal gene transfer, playing a noticeable role in the rapid spread of antibiotic resistance genes(ARGs). Broad host range plasmids are known to transfer rapidly to diverse bacteria in extracted soil bacterial communities when evaluated by filter mating incubation. However, the persistence and dissemination of broad range plasmid in natural soil has not been well studied. In this study, *Pseudomonas putida* with a conjugal plasmid RP4 were inoculated into a soil microcosm, the fate and persistence of both *P. putida* and RP4 were monitored by quantitative PCR and digital PCR. The concentrations of RP4 were lower than that of *P. putida*, and they both rapidly decreased within 20-day incubation. *P. putida* decayed at a significantly lower rate during subsequent incubation, however, no further decay of RP4 were observed, resulting in an elevated RP4/*P. putida* ratio (up to 10) after 60 days incubation, which implied potential conjugation of RP4 to soil microbiota. We further isolated RP4 recipient bacteria from the soil microcosms by fluorescence-activated cell sorting technique. Analysis of 16S rRNA gene sequences showed that these bacteria were affiliated to more than 15 bacterial phyla. Gram-negative Proteobacteria assumed absolute superiority, as represented in previous studies. . Remarkably, we detected two phyla of Archaea in transconjugant pool. These results suggest the environmental dispersion of plasmid mediated ARGs in natural

soil, and highlight the significant potential of broad range plasmids in transferring to wide range recipients, even including Archaea.

390A - Comparative network analysis revealing mechanisms of antibiotic resistance genes removal by leachate recirculation under different hydraulic loadings

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The wide dissemination of antibiotic resistance has become a pervasive global health threat. Landfill leachate, generated during treatment of municipal solid waste, has been an important hotspot of antibiotic resistance genes (ARGs). Given the feasibility and effectivity of leachate treatment by recirculation, this study aimed to assess the removal performance and mechanism of ARGs from leachate under different hydraulic loadings. The results showed that ARGs removal efficiencies were dependent on hydraulic loadings and ARGs types other than operating time, and the reactors operated with hydraulic loadings of 25 and 50 $L \cdot m^{-3} \cdot d^{-1}$ exhibited greater removal potential than the one with 100 $L \cdot m^{-3} \cdot d^{-1}$. Among the ARGs with great removal performance, genes *sul*2, *tet*Q, *aad*A1and bla_{CTX-M} were eliminated from leachate and refuse simultaneously, but for *tet*M, *erm*B, *mef*A, and *str*B, elimination and increased abundances were observed in leachate and refuse respectively. The results indicated that some types of ARGs were thoroughly eliminated from the system, and others were just transferred from leachate to refuse. Further investigation showed that bacterial community shift patterns were different in leachate and refuse, and the topology comparison analysis of co-occurrence network in leachate and refuse suggested a closer hosting relationship between ARGs and genera in refuse than leachate. Taxonomic category of host bacteria other than diversity of host genera shaped the ARGs removal, and the ARGs harbored in phyla Cyanobacteria, Tenericutes and Acidobacteria were more likely to be removed. These findings can potentially foster the understanding of ARGs removal mechanism in biological treatment processes.

391A - Insight into genomic plasticity of *Pseudomonas putida* KF715 which has unique properties in biphenyl-utilizing activity and genome instability

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Biphenyl-utilizing *Pseudomonas putida* KF715 has the unique properties in both catabolic activity and genome instability. Our previous studies revealed that DNA region encoding the biphenyl metabolisms (*bph*), was frequently deleted and transferred by conjugation to closely related *P. putida* strains. In this study, we first determined the complete nucleotide sequence of the KF715 wild type genome. Secondly, KF715 genome was compared with those of one KF715 defective mutant and two transconjugants together with several *P. putida* strains available in public

database. The gapless KF715 genome sequence revealed large number of plasmids. Southern blot analyses indicated the most of KF715 cell population carry the *bph* genes on chromosome and a small number carry it on plasmid pKF715A, and the pKF715A transfer by conjugation to recipient strains. These results suggested that pKF715A behave as Integrative and Conjugative Element (ICE). However, unlike to typical ICE, pKF715A carrying *bph* genes transfer by conjugation to recipient strains and did not integrate into chromosome in its recipient. Furthermore, by comparative genome analysis, a number of putative genetic elements which play a significant role in genome rearrangements were found in KF715. These genome data of KF715 provide insight into the genetic plasticity and adaptability of microorganisms in various ecological niches.

392A - Detection of macrolide resistance genes *mef*(C)-*mph*(G) on various mobile genetic elements in Taiwan waters

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Most of environmental bacteria are non-culturable. Hence, spreading of antibiotic resistance genes in environment has still been obscure. We found that the novel macrolide resistance genes, *mef*(C)-*mph*(G), were carried by two types of plasmids and chromosomal integrative conjugative element (ICE) in environmental isolates. This study aimed to reveal vector diversity for *mef*(C)-*mph*(G) in natural bacterial communities.

We collected pig manure leachate, river and coastal waters of Taiwan. Bacteria were captured on 0.2 µm pore-size-filters. Additionally, isolates were obtained from agar plate with erythromycin (16 µg/mL). Whole DNA was extracted from filters and pooled-colonies. We quantitated *mef*(C)-*mph*(G) and *traI* coded on plasmids pAQU1 and IncA/C and SXT/R391 family ICE (SRI) using quantitative PCR.

mef(C)-*mph*(G) were detected in both filter and colony DNAs from pig farm and river, whereas only in filter-DNA from marine fish farm. This suggests these genes are possessed by non-culturable assemblage in seawater. *mef*(C)-*mph*(G) were not detected in upper stream than pig farm. Pig farm maybe a source of this gene set. *traI* was detected in all areas; however, no positive correlation was observed with *mef*(C)-*mph*(G). This suggests *mef*(C)-*mph*(G) are carried by other unknown vectors than the plasmids and SRI targeted in this study.

Pig farm is one of sources of *mef*(C)-*mph*(G). Non-culturable bacteria possess this gene set in the sea. Unknown vectors would play a role to spread *mef*(C)-*mph*(G). This study newly showed *mef*(C)-*mph*(G) were widely disseminated in aquatic environment and harbored by various bacterial communities in each environment.

393A - Dissemination of *tet*(E) in *Aeromonas* strains isolated from a biofilm reactor treating oxytetracycline containing wastewater

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This study aimed to reveal the acquisition mechanism of antibiotic resistance gene in complex bacterial community under antibiotic pressure. An aerobic biofilm reactor was operated by adding increasing oxytetracycline concentrations (0, 0.1, 1, 5, 25, and 50 mg·L^{-1}). A total of 86 *Aeromonas* strains were isolated from different oxytetracycline stages, and phylogenetic analysis of 16s rDNA and housekeeping genes showed they belonged to the same clone. The minimal inhibitory concentration to oxytetracycline of *Aeromonas* was increased with the increase of the oxytetracycline concentrations, and *tet*(E) was the most prevalent tetracycline resistance genes, which was detected in 73% (63/86) of *Aeromonas* strains. *Tet*(E) was not found in *Aeromonas* genomes without oxytetracycline adding, and appeared to be inserted into the chromosome of *Aeromonas* strains obtained from low oxytetracycline concentration exposing stages (0.1, 1, and 5 mg·L^{-1}). Two types of chromosomal insertions, one mediated by phage-like integrase and the other one by a Tn*3* family transposon, were detected. When oxytetracycline concentration increased to 25 and 50 mg·L^{-1}, two types of plasmids (pAeca1-a and pAeca2) carrying *tet*(E) emerged in *Aeromonas* strains, where *tet*(E) gene was also located on Tn3 family transposons. Homologous analysis showed that the *tet*(E)-carrying Tn*3* family transposons found the chromosome and plasmids shared the same backbone (*tnpA* and *tnpR*) and similar passenger genes, suggesting that they may evolve from the same transposon. The results suggested that Tn*3* family transposons may play an important role in the horizontal dissemination of *tet*(E) in *Aeromonas* in biofilm bacterial communities facing increasing oxytetracycline concentrations.

394A - Human activity is the strongest determinant of antibiotic resistance genes in southwestern British Columbia

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The dissemination of antibiotic resistance genes (ARGs) from anthropogenic sources into the environment poses an emerging public health threat. The present study focused on putative antibiotic resistance and integrase genes present in the microbiome of agricultural, urban influenced and protected watersheds in southwestern British Columbia, Canada. A metagenomics approach and high-throughput quantitative PCR were used to screen for elements of resistance

including ARGs and integron-associated integrase genes (*intI*) in watersheds over a one-year period. There was a low prevalence of ARGs relative to the microbial population (<1%). Analysis of the metagenomic sequences detected a total of 60 elements of resistance including 48 ARGs, *intI1* gene, and quaternary ammonium compounds (*qac*) resistance genes across all watershed locations. The relative abundance and richness of ARGs was found to be highest in agriculture impacted watersheds compared to urban and protected watersheds. A downstream transport pattern was observed in the impacted watersheds during dry months. Similar to other reports, this study found a strong association between *intI1* and ARGs (e.g., *sul1*), an association which may be used as a proxy for anthropogenic activities. Chemical analysis of water samples for three major groups of antibiotics was below the detection limit. However, the high richness and gene copy numbers of ARGs in impacted sites suggest effects of effluents on microbial communities are occurring even at low concentrations of antimicrobials in the water column. Environmental factors such as land-use and water quality parameters accounted for 45% of the variability of ARGs observed in agriculture and urban watersheds.

395A - Optimizing EpicPCR: a Culture-Independent Method to Study Antimicrobial Resistance in the Environment

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The vast majority of microbial organisms are uncultivable; it is estimated that only 1 % of all bacteria can be cultivated *in vitro*. The remaining 99 % is called the microbial dark matter. Culture-independent, molecular methods are needed to characterize microbial dark matter as well as to define ‘who does what’ in the microbial world.

EpicPCR, short for Emulsion, Paired Isolation and Concatenation PCR, is an emulsion-based method, which can be used to answer the question of ‘who does what’ in a microbial community. In epicPCR a culture-free, emulsion-based fusion PCR -technique is used to link phylogenetical markers, such as 16S ribosomal RNA gene, to functional target genes, such as antibiotic resistance genes. This provides a throughput of hundreds of thousands of cells with relatively low costs. EpicPCR is a technique which has been used successfully in published research. However, the exact number of cells to give a positive signal in epicPCR is still in question.

My aim is to determine and present the sensitivity of epicPCR by using a transformed *Escherichia coli*-strain as an internal standard. I will also optimize the PCR-reagent concentrations used in epicPCR, as well as the PCR conditions including annealing temperatures and the amount of cycles used. With these optimization steps epicPCR could be applied more efficiently to learn about microbial dynamics and the ‘microbial dark matter’.

396A - Activation of integrative and conjugative element transfer and investigation of the element's core genes expression inside the donor cell

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Pseudomonas knackmussii B13 carries two copies of a mobile Integrative and Conjugative Element named ICE*clc*, which has served as model to understand ICE ecology and behavior. One of the particularities of ICE*clc* is its bistable activation in stationary phase, leading to 3-5% of individual cells in a population to develop transfer competence and becoming able to transfer the ICE*clc* by conjugation to new recipient cells.

The aim of our project is to unravel the network of regulatory decisions that leads to activation of the ICE*clc* transfer competence state in a subset of cells. Based on previous studies, all potential ICE*clc* promoters were identified and individually tested for bistable expression. This was done by fusing a promoterless *gfp* gene to each of the ICE*clc* promoters and integrating this construct in single copy in the chromosome of wild-type or mutant P. *knackmussii* B13 strains. We found 9 bistable promoters, situated in a strongly conserved region of ICE*clc*. Dual testing of each of those 9 promoters in conjunction with the *intB13* integrase promoter indicated that they are expressed in the same individual cell. From time-lapse microscopy we observed the onset of ICE*clc* promoter activation, suggesting a hierarchical network in expression of the ICE*clc* integrase and its conjugative system. We further show that most of the bistable promoters are dependent on *inr*R, a gene coding for a regulatory factor on ICE*clc*. The characterization of the various bistable promoters will help to further understand how bistability is exerted on promoter sequences.

397A - Spreading of multi drug resistant bacterial groups originated from animal husbandry into environments evaluated by the method based on MPN/MERFLP.

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Spreading of antibiotic resistant bacteria from animal husbandry is now becoming a serious public health problem to be in danger of their community-acquired infection through daily products, or vegetables, or underground water.

As diverse kinds of bacteria could acquire antibiotic resistances expressed by various resistant genes, it was difficult to evaluate their environmental risk by the unculture-based community analysis methods, such as DGGE or t-RFLP or clone library sequencing or pyro-sequencing. Because bacterial phylogenetic positions had no-relation to their resistance, which was irregularly evolved by acquiring diverse kinds of resistant genes, a development of suitable method has been required.

We thought that antibiotic resistant bacteria spreading into various environments could be monitored by identifying and quantifying the bacteria grown under application of antibiotics. Here we will present the results of our monitoring by using

our newly developed analysis method, by which numbers of each taxonomically different bacterial groups grown under antibiotics were calculated by MPN.

Diverse kinds of multi drug resistant bacteria, grown in LB medium containing streptomycin (25 mgl^{-1}), chloramphenicol (25 mgl^{-1}), and ampicillin (25 mgl^{-1}), were detected in liquid livestock feces *(*Actinobacteria, α-proteobacteria, *Pseudomonas spp.,* and The other γ-proteobacteria), and experimental fields (β-*proteobacteria,* α-proteobacteria, γ-proteobacteria, Actinobacteria ,and Firmicutes). The multi drug resistant bacteria with higher resistance (*Pesudomonas* spp.), grown under additional ciprofloxacin (25 mgl^{-1}), were detected in liquid livestock feces, and organic manure. More precise classification and each numbers of multi drug resistant bacterial groups calculated by MPN will be presented in the presentation.

398A - Exploring a cradle of multi-resistant bacteria –antibiotic resistance in soil and its link to different land use types and intensities

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Recent evidence has illustrated a connection between antibiotic resistance genes (ARGs) encoded by human pathogens and those detected in environmental microorganisms, underlining the requirement of deeper investigation of the environmental antibiotic resistance reservoir. This project focuses on the dynamics of the antibiotic resistance pool in soil with respect to different land use types and intensities.

A microcosm experiment was conducted, where soils with different land use histories were treated with different mixtures of antibiotics, commonly used in veterinary medicine. Moreover, function-based screenings of metagenomic libraries, constructed from isolated soil DNA, were performed to discover novel antibiotic resistance genes and differences in richness of resistance mechanisms associated with land use intensity.

First results indicated shifts in soil bacterial community composition in response to treatment with an array of antibiotics. In addition, novel potential resistance genes in soil were identified in grassland and forest samples.

399A - The risk and control of antibiotic resistance genes in landfill system

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Landfills are reservoirs of antibiotics, heavy metals, disinfectants and other emerging contaminants such as antibiotic resistant genes, and attracted serious concerned of the research recently. The antibiotics and antibiotic resistant genes were investigated at seven different landfills cross the China using modern analysis technology in this study, the results showed that fluoroquinolones (average 268.56

μg/kg) and tetracyclines (average 68.66 μg/kg) were dominated antibiotics in landfill refuse respectively, and macrolides (average 2,980.73 ng/L) and sulfonamides (average 3,470.43 ng/L) were the most one in the leachate, respectively. While sul1, ermB and intl1 were major antibiotic resistant genes both in landfill leachates and refuses with 7.61, 6.13, 7.31 log copies/ng DNA in landfill refuse, and 7.22, 7.14, 7.12 log copies/ng DNA, respectively in landfill leachates. The antibiotics levels were consistently lower in refuse and leachates from older landfills, while antibiotic resistant genes increased significantly with landfill age, whereas antibiotic resistant genes in leachates were more associated with mobile genetic elements, which implied greater antimicrobial resistance exposure risks around older landfills. Meanwhile, the abundance of antibiotic resistant genes, antibiotic-resistant bacteria and mobile genetic elements decreased along with the leachate treatment process by 1- 2 orders of magnitude, whereas the average abundance of mobile genetic elements in receiving water significantly increased from 6.8 ± 1.4 to 7.5 ± 0.5 log (copies/ng DNA), which co-occurred with an elevated level of antibiotic resistant genes and antibiotic-resistant bacteria, showing the traditional treatment process be promoted if antibiotic resistant genes control efficiently before discharging to the nature environment.

400A - Drinking well water contaminated by antibiotic resistance genes (ARGs) in the co-livestock farm

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Livestock farm has been considered as a propagating source of antibiotic resistance gens (ARGs) due to the discharge of animal wastes carrying resistant bacteria, however, not much information available on the ARGs in well water which animal direct intake. To investigate the dissemination of ARGs in well water and assess the impact of rainfall on the ARGs, we analyzed the abundance and diversity of ARGs in a long term co-livestock feedlot farm with the dry and wet season. The results revealed that nineteen ARGs were detected in well water and the total concentrations of target ARGs (∑ARGs) were increased up to 4.32 × 10^4 copies/mL and 4.73× 10^5 copies/mL in dry season and wet season respectively. The piggery wastewater and fishponds showed about 10^9 copies/mL and 10^6 copies/mL of ∑ARGs, respectively. Biogas digester and lagoon treatment process showed limit removal of ∑ARGs and reduced its order of magnitude. Lagoon and fishpond through it harder to control the dissemination of ARGs pollution in the open form and stream assisted it especially during wet season. Affected by rainfall, the concentrations of ARGs in samples increased, and the correlation between surface water and groundwater became closer. These results suggest that livestock farm surrounding environment and direct drinking water source was bearing serious ARGs contamination, which could pose ecological risk and food safety problems. During this investigation, optional *tet*B should be a reference factor forthcoming evaluation of tetracycline resistance genes in well water.

401A - Large-scale biogeographical patterns of bacterial antibiotic resistome in water bodies of China

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Antibiotic resistance genes (ARGs) are widespread in aquatic environments, but we know little about their biogeographical distribution and occurrence at national scales. Here we analyzed the patterns of ARGs from 42 natural lakes/reservoirs across China using high-throughput approaches. The multidrug resistance genes and efflux pump were the major ARGs and resistance mechanism in these lakes/reservoirs, respectively. Although, the absolute abundance (gene copies/L) of ARGs in the south/central lakes/reservoirs was similar with the north lakes/reservoirs, the relative abundance of ARGs (ARGs/16S rRNA gene copy number) was higher in the south/central lakes/reservoirs than in the north (mainly because of the aminoglycoside and multidrug resistance genes). Human activities strongly correlated with the relative abundance of ARGs. The composition of ARGs in the south/central Chinese lakes/reservoirs was separated from that in the north, and ARGs showed a distance-decay relationship. Anthropogenic factors had the most significant effects on this spatial distribution of ARG composition, followed by the spatial, physicochemical and bacterial factors. These indicate that the ARGs exhibited biogeographical patterns and that multiple ecological mechanisms - such as local environmental selection (human activities and local physicochemical parameters) and dispersal limitation - influence distribution of ARGs in these waters. In general, our results provide a valuable ecological insight to explain the large-scale dispersal patterns in ARGs, thereby having applications for both public health and environmental management.

402A - Whole Genome Sequencing resistome analysis of Multi Drug Resistant *Shewanellaceae* and *Vibrionaceae* isolates collected from the Adriatic Sea (Italy)

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Resistance to carbapenems and third generation cephalosporins is a global concern for the treatment of Multi Drug Resistant Gram negative bacterial infections. In this study, we analyzed by Whole Genome Sequencing technology the resistome and mobilome of 12 Multi Drug Resistant *Shewanellaceae* and *Vibrionaceae* strains with the aim of detecting possible new resistance genes in the aquatic environment. Forty-one marine strains were screened for the presence of beta-lactamases and carbapenemases. We used the enzymatic Blue-Carba Test and the ability of bacteria to grow on chromID ESBL medium. Strains genotyping was performed by Multiplex PCR targeting the most clinically relevant genes (bla_{KPC}, bla_{VIM}, bla_{NDM}, bla_{IMP}, bla_{OXA-48}) and their variants. Although all the strains resulted negative by Multiplex PCR, eleven proved positive for the enzymatic activity assay and their genomes were whole sequenced using the Illumina NextSeq® 500 platform. Bioinformatic analysis revealed the presence of sequences with high homology

(98% - 100%) to genes that confer resistance to β-lactams (*bla*$_{ampC}$, *bla*$_{PSE-4}$, *bla*$_{OXA-55}$), quinolones (*qnrVC6, qnrA3, qnrA7*) and oxytetracycline (*tet34, tet35*). Members of Major Facilitator System and Resistance-Nodulation-cell Division efflux pumps superfamilies were found. Insertion Sequence transposases such as IS*200*/IS*605*, IS*630*, ISL*3*, IS*110* and IS*481* were detected, whereas an integron integrase was found in 7 strains. To conclude, Whole Genome Sequencing is a good technology to investigate antimicrobial resistance genes in the aquatic environment. The location of these genes in Mobile Genetic Elements could favour their spread among the microbial communities and human pathogens encountered in this ecological niche.

403A - Manure fertilization drives co-occurrence of antibiotic and metal resistance genes across paddy fields

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Antibiotics and heavy metals are supposed to be the major drivers of the blooming and spread of antibiotic resistance genes (ARGs). However, the profile of metal resistance genes (MRGs) and the co-occurrence relationships with ARGs in fields remain largely unknown. In this study, 66 manure samples and 286 soils samples were taken from paddy fields across a range of soil types and climates in the east China. High throughput functional gene array and Illumina sequencing were used to investigate the distribution and co-occurrence patterns of ARGs and MRGs of selected samples. In total, 10,206 coding sequences from 18 functional gene families related to antibiotic resistance and 25,312 coding sequences from 101 functional gene families related to metal resistance were detected. The results indicated that manure fertilization increased the abundance of both ARGs and MRGs. Similar distribution pattern and significantly positive correlations were found between ARGs and MRGs. Network associations between ARGs and MRGs were more complex in soils with manure fertilization compared with inorganic fertilization, which suggested that MRGs might stimulate bloom of ARGs with conjugative mobile genetic elements-mediated horizontal gene transfer and some transcriptionally linked regulatory systems. Acidobacteria, Actinobacteria, Firmicutes and Proteobacteria were predominant potential hosts for the coexisted resistance. Structural equation models demonstrated that manure inputs were main drivers of soil ARGs and MRGs co-occurring associations. Negative effects of temperature on the associations showed directly and indirectly through soil bacteria. These findings provide new insights into the co-occurrence of ARGs and MRGs and the controlling mechanism.

404A - Feed additives shift gut microbiota and enrich antibiotic resistance in swine gut

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Antibiotic resistance genes (ARGs) are emerging environmental contaminants posing a threat to public health. Antibiotics and metals are widely used as feed additives and could consequently affect ARGs in swine gut. In this study, high-throughput quantitative polymerase chain reaction (HT-qPCR) based ARG chip and next-generation 16S rRNA gene amplicon sequencing data were analyzed using multiple statistical approaches to profile the antibiotic resistome and investigate its linkages to antibiotics and metals used as feed additives and to the microbial community composition in freshly collected swine manure samples from three large-scale Chinese pig farms. A total of 146 ARGs and up to 1.3×10^{10} total ARG copies per gram of swine feces were detected. ARGs conferring resistance to aminoglycoside, macrolide-lincosamide-streptogramin B (MLSB) and tetracycline were dominant in pig gut. Total abundance of ARGs was positively correlated with in-feed antibiotics, microbial biomass and abundance of mobile genetic elements (MGEs) ($P < 0.05$). A significant correlation between microbial communities and ARG profiles was observed by Procrustes analysis. Network analysis revealed that Bacteroidetes and Firmicutes were the most dominant phyla co-occurring with specific ARGs. Partial redundancy analysis indicated that the variance in ARG profiles could be primarily attributed to antibiotics and metals in the feed (31.8%), gut microbial community composition (23.3%) and interaction between feed additives and community composition (16.5%). These results suggest that increased levels of in-feed additives could aggravate the enrichment of ARGs and MGEs in swine gut.

405A - Identifying mobile plasmids *in-situ* in various ecosystems

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Microbes have various ways of communication, one of which involves transferring plasmids between and among bacterial species- a process termed horizontal gene transfer. This extraordinary phenomenon enables rapid bacterial evolution and gain of function, such as antibiotic resistance. Thus, an increase in horizontal gene transfer rates can be critical in determining the functionality of a given bacterial population.

To investigate the influence of plasmid mobility in various environments, it is essential to identify these plasmids within their host cells (*in-situ*). A new modification of the well-known Fluorescent *in-situ* Hybridization method allows us to do so, by hybridizing short probes to plasmids of interest. This Catalyzed Reporter Deposition Single Molecule Fluorescence in-situ Hybridization method, combines enzymatic signal amplification, which increases sensitivity, using small oligonucleotide probes, which are known to increase specificity, to enable the labeling of small and low-copy number plasmids, by using as little as 15 small probes.

Comparing images of labeled mobile plasmids and examining the differences in their distribution throughout microbial communities of different environments, may elucidate their role in these ecosystems.

406A - Distribution of antibiotic resistances and antibiotic resistant bacteria in wastewater treatment plants assessed by metaproteomics

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The massive use of antibiotics in human medicine as well as animal breeding leads to a growing input of antibiotics and antibiotic resistant bacteria (ARB) into the environment. Urban wastewater treatment plants (UWTP) have been considered as hot spots for the emergence and dissemination of ARB. The high bacterial density in these facilities promotes horizontal gene transfer and therefor the exchange of antibiotic-resistance determinants between pathogenic and non-pathogenic bacteria. Using a metaproteomics approach, we aim at the identification and quantification of metabolically active and antibiotic-resistant bacteria as well as resistance determinants at different sites of the UWTP.

Samples from different stages of sewage treatment were analyzed by GeLC-MS/MS. For protein identification sequence databases based on metagenomic data were created. Antibiotic resistance proteins were predicted using the Resfams database. In addition, culture-dependent methods were applied to determine the proportion of ARB.

Bacterial community profile changes during sewage treatment. Members of the human gut microbiome and bacteria related to infections of the gastro-intestinal/urinary tract that are known to carry antibiotic resistances dominate the inlet community. Activated sludge processes add bacteria involved in nutrient degradation. Treated wastewater contains significantly decreased bacterial numbers but still includes ARB and pathogens. Accordingly, proteins associated to antibiotic resistances are found at all stages of wastewater treatment.

Sewage treatment leads to reduction but not clearance of ARB and potential pathogens. Their release into the environment could promote the spread of antibiotic resistances.